201-16127B

201-14906B

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: **RECLAIMED SUBTANCES: NAPHTHENIC ACID**

Summary prepared by: **American Petroleum Institute**

Date of last Update: **DECEMBER 15, 2003**

Number of pages: **40**

Reliability of data included in this summary has been assessed using the approache described by Klimisch, et al.

Klimisch, H.J., Andreae, M. and Tillman, U (1997)
A systemic approach for evaluating the quality of experimental toxicological and exotoxicological data.
Regulatory Toxicology and Pharmacology 25: 1-5

1. General Information

1.1 GENERAL SUBSTANCE INFORMATION

Substance Type: Naphthenic Acids

Physical status: Naphthenic acid fractions are oily liquids. The salts may be liquid or solid. Naphthenic acids (CASRN 1338-24-5) are classified as monobasic carboxylic acids of the general formula RCOOH, where R represents the naphthene moiety consisting of cyclopentane and cyclohexane derivatives. Naphthenic acids are composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids, with smaller amounts of acyclic aliphatic acids. The cycloaliphatic acids include single and fused multiple cyclopentane and cyclohexane rings. The carboxyl group is usually attached to a side chain rather than directly to the ring. Aromatic, olefinic, hydroxy and dibasic acids are present as minor components.

Naphthenic acids recovered from refinery streams occur naturally in the crude oil and are not formed during the refining process. Heavy crudes have the highest acid content, and paraffinic crudes usually have low acid content. Naphthenic acids are obtained by caustic extraction of petroleum distillates, primarily kerosene and diesel fractions.

2. Physical and Chemical Data

2.1 MELTING POINT

Test Substance: Naphthenic Acids, commercial mixtures

Method: Not stated

Year (Guideline): Not stated

Type (test type): Not stated

GLP: Unknown

Test Conditions: Unknown

Results:

-35 °C to +0 °C	Ref (1)
-35 °C to +2 °C	Ref (2)
+30 °C	Ref (3)

Remark: Values cited represent ranges of melting points cited in product literature data and Material Safety Data Sheet for commercial naphthenic acid products.

Source:

(1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

(2) AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

(3) Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.

Reliability: (4) Not assignable. Original source data were not available for review.

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method/Guideline: Calculated values using MPBPWIN Version 1.40, a subroutine of the computer program EPIWIN Version 3.10

Year (guideline): 2000

Type (test type): Not applicable

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10

Results:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Melting Point, °C
1-ring cyclopentane	16	254	117
1-ring cyclohexane	21	325	155
2-ring cyclopentane	17	266	127
2-ring cyclohexane	21	323	157
3-ring cyclohexane	17	264	128
3-ring cyclohexane	21	321	160
4-ring cyclohexane	17	262	131
4-ring cyclohexane	21	319	156

Remark:

Substances in this category do not have a specific melting point but a range of melting points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual melting point ranges will vary dependent upon their constituent composition.

Melting point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated melting points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products, as evidenced by comparing the estimated melting point values to those cited in product literature and MSDS data (SocTech, S.A. 2003; AGS Chemicals Limited. 2003; Mallinckrodt Baker, Inc. 1997).

Source:

U.S. EPA. 2000. API (Estimation programs interface) suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

Reliability:

(2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of melting point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

2.2 BOILING POINT

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Not stated

Year: Not stated

Type: Not stated

GLP: Not stated

Year (study performed): Not stated

Test Conditions: Not stated

Results:

250 °C to 350 °C	Ref (1)
140 °C to 200 °C	Ref (2)
200 °C to 370 °C	Ref (3)

Remark: Values reported vary widely due to varied composition of the hydrocarbon mixture in naphthenic acids. Values given represent various commercial preparations of naphthenic acids.

Source:

(1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

(2) AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

(3) Brient, J.A., P.J. Wessner, and M.N. Doyle. 1995. Naphthenic Acids. In: Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, Inc.

Reliability: (4) Not assignable

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, MPBPWIN V1.40 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10

Results: Boiling point values for various cycloaliphatic carboxylic acids in naphthenic acid mixtures are:

Compound	Estimated Boiling Point, °C
C7 cyclohexane	233
C9 dicyclopentane	266
C10 cyclopentane	284
C11 cyclohexane	301
C13 dicyclopentane	326
C14 cyclopentane	340
C15 cyclohexane	352
C17 dicyclopentane	373
C17 tricyclohexane	375

Remark: Substances in this category do not have a specific boiling point but a range of boiling points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual boiling point ranges will vary dependent upon their constituent composition.

Boiling point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated boiling points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Reliability: (2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of boiling point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

2.4 VAPOR PRESSURE

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, MPBPWIN V1.40 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, vapor pressures were calculated by MPBPWIN, V1.40, EPIWIN V3.10

Results: Estimated vapor pressures for various naphthenic acid compounds:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Vapor Pressure, Pa
1-ring cyclopentane	16	254	1.8×10^{-3}
1-ring cyclohexane	21	325	1.5×10^{-5}
2-ring cyclopentane	17	266	4.8×10^{-4}
2-ring cyclohexane	21	323	1.5×10^{-5}
3-ring cyclohexane	17	264	4.2×10^{-4}
3-ring cyclohexane	21	321	1.4×10^{-5}
4-ring cyclohexane	17	262	1.6×10^{-5}
4-ring cyclohexane	21	319	4.4×10^{-4}

Remark: A search for pressure values of naphthenic acids failed to uncover reliable information. Product literature data provided narrative phrases such as "very low" or "not applicable" when describing the vapor pressure characteristic for commercial products (SocTech, S.A., 2003; AGS Chemicals Limited. 2003). To gain an understanding of vapor pressure characteristics of naphthenic acids, various hydrocarbon acidic structures reported by Rogers et al. (2002) to predominate in naphthenic acids were estimated for vapor pressure using the EPIWIN© computer model (U.S. EPA 2000).

The vapor pressure of complex mixtures is equal to the sum of the vapor pressures of the individual constituents in their pure form times their mole fraction in the mixture (Raoult's Law). Therefore, the total vapor pressure of a complex mixture of naphthenic acids will depend on the proportion of different molecular weight constituents making up the mixture. It is estimated from vapor pressure modeling that representative individual naphthenic acid molecules will have vapor pressure values near or below the measurable limits cited in standard reference guidelines (OECD Guideline 104, Vapor Pressure; OECD, 1995). Hence, based on Raoult's Law, the total vapor pressure of naphthenic acids is expected to be exceedingly low.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

OECD (Organization for Economic Cooperation and Development). 1995. OECD Guideline 104, Vapor Pressure. OECD, Paris, France.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

Reliability: (2) Reliable with restrictions
Estimated vapor pressures were obtained from a validated computer program.

2.5 PARTITION COEFFICIENT

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, KOWWIN V1.66 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, vapor pressures were calculated by KOWWIN, V1.66, EPIWIN V3.10

Results: Tabulated values for various naphthenic acid molecules are:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Log Kow
1-ring cyclopentane	16	254	6.7
1-ring cyclohexane	21	325	9.2
2-ring cyclopentane	17	266	6.3
2-ring cyclohexane	21	323	8.3
3-ring cyclohexane	17	264	5.4
3-ring cyclohexane	21	321	7.3
4-ring cyclohexane	17	262	6.5
4-ring cyclohexane	21	319	5.1

Remark: No partition coefficient measurements were found for naphthenic acids. Therefore, partition coefficients for a range of molecular weight naphthenic acids were estimated using the EPIWIN© computer model (U.S. EPA 2000). The partition coefficients reported here span the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected, however, that the lowest molecular weight structures will have the lowest partition coefficients of the compounds in the complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures of molecular weights below 250 will likely show log Kow values lower than those estimated here.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

Reliability: (2) Reliable with restrictions
Estimated vapor pressures were obtained from a validated computer program.

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in: Water

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, WSKOWWIN V1.40 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, water solubility values were calculated by WSKOWWIN, V1.40, EPIWIN V3.10

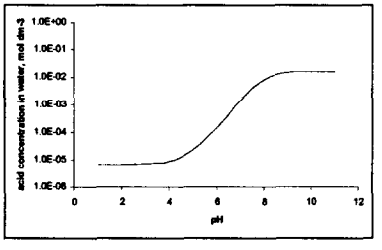
Results: Tabulated estimates at 25°C for various naphthenic acid molecular structures are:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Water Solubility, mg/l
1-ring cyclopentane	16	254	0.11
1-ring cyclohexane	21	325	0.0003
2-ring cyclopentane	17	266	0.19
2-ring cyclohexane	21	323	0.002

3-ring cyclohexane	17	264	1.2
3-ring cyclohexane	21	321	0.01
4-ring cyclohexane	17	262	0.08
4-ring cyclohexane	21	319	2.1

Remark:

No water solubility measurements were found for naphthenic acids, but their dissociation equilibrium in aqueous systems provides a general understanding of their behavior. These compounds exist as weak acids, with most pKa values being reported at about 5 (Havre, 2002). At low pHs, these compounds exist in their undissociated form and tend to partition onto solids (Rogers et al., 2002). At high pHs, they exist in their dissociated form and become more mobile (Havre, 2002). The following plot shows a theoretical model of the concentration of the acid in the water phase with water pH. This relationship is used as the basis for extraction of naphthenic acids from crude oil, where an alkaline hot water extraction process is used (CEATAG 1998; Brient et al., 1995). However, solubility does not follow an exact acid/base equilibrium, and the equilibrium between oil and water becomes increasingly complex as pH rises. This is due to the tendency of these substances to form micelles and reversed micelles at alkaline pHs. In this system, the existence of 4 or 5 isotropic phases can be observed, making exact solubility measurements difficult (Havre, 2002).



from Havre, 2002

To gain an overview of the water solubility of a range of molecular weight naphthenic acids, the EPIWIN© computer model (U.S. EPA 2000) was used to generate solubility estimates for different molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected that the lowest molecular weight structures will have the greatest water solubility of the compounds in complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures having molecular weights below 250 will likely show water solubilities greater than those estimated here.

Source:

U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Havre, T.E. 2002. Formation of calcium naphthenate in water/oil systems, naphthenic acid chemistry and emulsion stability. Ph.D. Thesis, Department of Chemical Engineering, Norwegian University of Science and Technology, Trondheim, Norway. October 2002.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

Brient, J.A., P.J. Wessner, and M.N. Doyle. 1995. Naphthenic acids. In: Kroschwitz, J.I. (ed.). Encyclopedia of Chemical Technology, Vol. 16, 4th ed. John Wiley & Sons, Inc., New York. pp 1017 – 1029.

Reliability: (2) Reliable with restrictions
Estimated water solubility values were obtained from a validated computer program.

2.14 ADDITIONAL REMARKS

Memo: Water solubility of naphthenic acids

Remark: Values of water solubility reported in product literature data have varied widely. CEATAG (1998) reported water solubility values of one commercial product to range from 70 mg/l at pH 0.91 to 5040 mg/l at pH 9.16. Other product data sources for water solubility report narrative phrases such as "very low water solubility" (SocTech S.A., 2003), "not applicable" (Mallinckrodt Baker Inc., 1997), or "only slightly soluble in water" (AGS Chemicals Limited, 2003).

Source: CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm

Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.

Reliability: (4) Not assignable. Data were obtained from secondary literature sources.

3. Environmental Fate Data

3.1.1 PHOTODEGRADATION

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculations by EPIWIN© V3.10; Subroutine AOPWIN V1.90.

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, photodegradation potential was calculated by AOPWIN, V1.90, EPIWIN V3.10

Results:

Type	Carbon Number	Molecular Weight	Half Life (days)
1-ring cyclopentane	16	254	0.6
1-ring cyclohexane	21	325	0.4
2-ring cyclopentane	17	266	0.5
2-ring cyclohexane	21	323	0.3
3-ring cyclohexane	17	264	0.3
3-ring cyclohexane	21	321	0.3
4-ring cyclohexane	17	262	0.3
4-ring cyclohexane	21	319	0.3

Remark: AOPWIN V1.90 calculates atmospheric oxidation rate constants between photochemically produced hydroxyl radicals and organic chemicals. These rate constants are then used to calculate half lives for those compounds based on average atmospheric concentrations of hydroxyl radicals and ozone. Atmospheric oxidation rates were calculated for a range of molecular structures covering a range of molecular weights and ring structures that were reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002).

Although the low vapor pressures of these base oils indicate that volatilization will not be a very significant fate process, oxidation half-lives indicate that any vapors emitted to the troposphere would be rapidly oxidized and not persist in the atmosphere.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

	Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.
Reliability:	(2) Reliable with restrictions Estimated water solubility values were obtained from a validated computer program.

3.1.2 STABILITY IN WATER

Remark:	Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). The chemical components found in the materials that comprise the gas oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.
Source:	Harris, J.C. 1982. Rate of hydrolysis. In; Handbook of Chemical Property Estimation Methods. W.L. Lyman, W.F. Reehl, and D.H. Rosenblastt, eds. Mcgraw-Hill Book Co., New York, NY.

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Test Substance:	Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)
Method:	Level 1 Fugacity-Based Environmental Equilibrium Partitioning Model (Version 2.11)
Year:	2000
Type:	Estimation, computer model
GLP:	Not applicable
Year (study performed):	Not applicable
Test Conditions:	The EQC Level I is a steady state, equilibrium model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment.
Results:	Air / Water / Soil / Sediment / Suspended Sediment / Biota
	Type (C-number)(Molecular Weight)
	Distribution In:

Air	Water	Soil	Sediment	Suspended Sediment	Biota
1-ring cyclopentane (C16)(254)					
<0.1	<0.1	98	2	<0.1	<0.1
1-ring cyclohexane (C21)(325)					
<0.1	<0.1	98	2	<0.1	<0.1
2-ring cyclopentane (C17)(266)					
<0.1	<0.1	98	2	<0.1	<0.1
2-ring cyclohexane (C21)(323)					
<0.1	<0.1	98	2	<0.1	<0.1
3-ring cyclohexane (C17)(264)					
<0.1	0.4	97	2	<0.1	<0.1
3-ring cyclohexane (C21)(321)					
<0.1	<0.1	98	2	<0.1	<0.1
4-ring cyclohexane (C17)(262)					
<0.1	<0.1	98	2	<0.1	<0.1
4-ring cyclohexane (C21)(319)					

Remark: Multimedia distribution was calculated for a range of naphthenic acids covering predominant molecular weight and ring structures of such constituents found in Athabasca oil sands extracts (Rogers et al., 2002). The principle distribution of these constituents following an environmental release would be to soil and/or sediment, with overwhelming partitioning to soil.

Source: Mackay, D. 1991. Multimedia environmental models; The fugacity approach Lewis Publ. CRC Press, Boca Raton, Florida.

Reliability: (2) Reliable with restrictions
Estimated environmental distribution was obtained from a validated computer program.

3.5 BIODEGRADATION

Remark: No standardized testing for ready or inherent biodegradation was found for naphthenic acids. Results of relevant scientific journal articles on the biodegradability of naphthenic acids are reviewed in Section 3.8

3.8 ADDITIONAL REMARKS

Memo: Biodegradation of naphthenic acids

Remark: Herman et al. (1993) conducted four experiments on the biodegradation of specific cycloalkane carboxylic acids:

Experiment No. 1. Biodegradation of four naphthenic acid compounds (cyclopentane carboxylic acid, CCP; cyclohexane carboxylic acid, CCH; 1-methyl-1-cyclohexane carboxylic acid, 1MCCH; and 2-methyl-1-

cyclohexane carboxylic acid, 2MCCH) was measured in pore water from Athabasca oil sands tailings ponds. The purpose of the tailings ponds was to serve as a settling basin to separate solids from liquid generated during the extraction of acidic compounds from bitumen. Therefore, the tailings ponds were considered to harbor indigenous microorganisms adapted to naphthenic acids. The collected pore water was centrifuged and filtered and served as the nutrient medium. Inoculum was 0.5 ml of the original oil sands tailings sample. Duplicate flasks containing 30 ml of medium were spiked with 1-ml aliquots of stock solutions of the different naphthenic acids to achieve a final concentration of 1000 mg/l. Test flasks received the inoculum and control flasks received inoculum in which the microbes had been heat-killed. One set of duplicate flasks received a nutrient addition in the form of NH_4NO_3, K_2HPO_4, and KH_2PO_4 to a final concentration of 0.2 g/l of each compound. The flasks were incubated at room temperature on a rotary shaker. After 0, 3, 6, 9, 16, 26, and 40 days, a 3-ml sample was removed, centrifuged, and filtered through a 0.2 micron syringe filter. The samples were analyzed for the test compounds by gas chromatography equipped with a flame ionization detector. Peak areas were converted to concentration using a calibration curve for each compound.

Results of Experiment 1. The bacterial populations of oil sands tailings was shown to have the metabolic capability of degrading carboxylated cycloalkanes as shown in the following table of results.

Percent Remaining

	CCP		CCH		1MCCH		2MCCH	
Day	NP-	NP+	NP-	NP+	NP-	NP+	NP-	NP+
0	100	42	100	68	100	100	100	100
6	100	5	100	12	100	100	100	100
10	100	0	100	1	100	100	100	100
16	100	0	100	0	100	100	100	100
26	100	0	100	0	100	100	100	49
40	100	0	100	0	100	100	100	0

Using tailings pond water as a growth medium, degradation of CCP, CCH, and 2MCCH was achieved only if nutrients were added to the medium. CCP and CCH were degraded rapidly, within one week, while methylated carboxylic acids were more resistant to biodegradation. 2MCCH was degraded within 40 days, but no degradation was observed for 1MCCH.

Experiment No. 2. Triplicate tailings pond microcosms were created using 200 ml of the tailings sample (as inoculum and medium) in 500-ml Erlenmeyer flasks closed with cotton stoppers. A filter-sterilized solution of CCP and 1MCCH was added to each microcosm for a final concentration of 1000 mg/l. Sterile controls were autoclaved and also spiked with the test compounds. Microcosms were incubated at room temperature on a rotary shaker. After 1, 2, 3, 4, 6, and 9 weeks, samples were removed and analyzed for CCP and 1MCCH by GC.

Results of Experiment No. 2. Biodegradation of CCP was complete within the first week. No biodegradation of 1MCCH was evident after six weeks. At the six-week period, nitrogen and phosphorus was added

whereby complete biodegradation of 1MCCH was noted following between the 6 and 9-week sampling. No 1MCCH was measured at 9 weeks. Neither CCP nor 1MCCH was degraded in the control microcosms.

Experiment No. 3: Tailings pond bacteria were isolated on agar plates and colony types were examined for their ability to utilize carboxylated cycloalkanes as their sole carbon source. Individual colonies were inoculated into a solution of carboxylated cycloalkanes (1000 mg/l) in modified Bushnell and Haas (MGH) minimal salts medium. The ability of the isolate to metabolize the carbon source was monitored by GC analysis. In a second part to this experiment, a carboxylate-degrading mixed bacterial culture was enriched from the tailings pond sample using standard procedures. The mixed culture was maintained on a mixture of CCP, 1MCCH, and 2MCCH (500 mg/l each) in MBH with yeast extract (1000 mg/l) added as a supplemental carbon source.

Results of Experiment No. 3. Of 10 separate colony types isolated from oil sands tailings, one colony type was found to utilize CCP and CCH as its sole carbon source. The isolate was a Gram negative, non-motile, catalase positive, oxidase negative, non-fermenting, aerobic rod, and was identified as an *Acinetobacter* sp. The isolate rapidly degraded CCP and CCH, with complete loss of substrate from the medium within 2 weeks of incubation. However, this isolate was unable to degrade methyl-substituted cyclohexane carboxylic acids. The mixed bacterial culture enriched from the tailings pond sample on a mixture of carboxylated cycloalkanes was found to degrade 1MCCH and 2MCCH, but only when the medium was supplemented with yeast extract. After a 2-week incubation period, the mixed culture had degraded 100% of the 1MCCH and 67% of the 2MCCH.

Experiment No. 4. Radiolabeled hexadecane was spiked into the maltene fraction of pure bitumen. Hexadecane mineralization experiments were performed using 5 ml of oil sands tailings in 60-ml serum vials and inoculated with 10 ul of spiked maltene. One set of vials received nutrient addition as described before. Sterile controls were autoclaved before the addition of the labeled hydrocarbon. Mineralization was determined from triplicate vials after 5, 10, 16, 27, and 40 days using the closed-loop trapping system. Radioactivity was measured using a scintillation cocktail and a Beckman LS8000 scintillation counter.

Results of Experiment No. 4. The results of hexadecane mineralization within oil sands tailings showed that the biodegradation of an n-alkane was nutrient limited. Percent biodegradation reached 50% by day 16 and maintained a plateau through day 40.

Conclusions. This study showed the potential for biodegradation of naphthenic acids by investigating the biodegradation of both carboxylated cycloalkanes and hexadecane. Although natural naphthenic acids present in oil sands tailings have greater structural complexity than the compounds examined in this study, the results show the potential for both for biodegradation of the alkyl side chain and the carboxylated cycloalkane ring components of naphthenic acids. Biodegradation potential was reduced by methyl substitution on the

cycloalkane ring, although these compounds could be degraded with the addition of mineral nutrients.

Source: Herman, D.C., P.M. Fedorak, and J.W. Costerton. 1993. Biodegradation of cycloalkane carboxylic acids in oil sand tailings. Can. J. Microbiol. 39:576-580.

Reliability: (2) Reliable with restrictions. The report was a well-documented study that meets basic scientific principles.

Memo: Biodegradation of cycloalkane carboxylic acids in oil sand tailings

Remark: Herman et al. (1994) investigated the ability of microbial populations indigenous to oil sands tailings to biodegrade solutions of natural naphthenic acids from oil sands tailings and commercial naphthenic acid sodium salts (Kodak Chemicals).

Four experiments were run:
1) Evaluation of mineralizaton of naphthenic acids sodium salts (NAS) and oil sands tailings extracts of naphthenic acids (TEX),
2) Evaluation of mineralization of four model naphthenic acid compounds, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA) 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and *trans*-4-pentylcyclohexane carboxylic acid (4PCCA),
3) Gas chromatographic analysis of NAS and TEX biodegradation, and
4) Respirometry measurements of cyclohexane pentanoic acid, NAS, and TEX in tailings microcosms.

Test Substances: Test substances used in the four experiments included the following materials: 1) Tailings water extract (TEX), 2) commercial sodium naphthenate mixture (NAS), and 3) pure compound naphthenic acids, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA), 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and *trans*-4-pentylcyclohexane carboxylic acid (4PCCA).

Inoculum: Inoculum used in the biodegradation experiments was NAS- and TEX- degrading enrichment cultures derived from oil sands tailings water. These cultures were created by diluting a 10-ml sample of oil sands tailing into 90 ml of mineral salts medium that contained either NAS (100 mg/l) or TEX (1:50 dilution). The mineral salts medium was modified Bushnell-Haas medium. Successive transfers 1% v/v) of the enrichment culture into fresh NAS- to TEX-containing medium were on monthly basis and incubated at room temperature on a gyratory shaker (100 rpm). The viable cell number within each enrichment culture was estimated using the plate count technique.

Experiment No. 1. A measurement of CO_2 production was used to evaluate the ability of the enrichment cultures to mineralize components within both the NAS and TEX mixtures. Mineralization experiments were performed using 60-ml serum bottles containing 15 ml of growth medium. The growth medium consisted of sterilized mineral salts medium with NAS (100 mg/l) or TEX (1:20 and 1:50 dilutions) as the sole carbon source. Dissolve organic carbon analyses showed that 100 mg/l of NAS contained 60 mg C/l, while 1:20 and 1:50 dilutions of TEX contained 50 and 21 mg C/l, respectively. The serum bottles were

inoculated with 0.15 ml of either the NAS-degrading or the TEX-degrading enrichment culture, sealed with rubber stoppers, and incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 6-day intervals over 24 to 30 days, three inoculated bottles and one control (inoculated but lacking NAS or TEX) were acidified to pH <2 using 1 ml of 1M H_2SO_4 to convert all forms of inorganic carbon into CO_2. A 0.5 ml headspace sample from each bottle was analyzed for CO_2 content by gas chromatography. Mineralization of the organic substrate was first corrected for the amount of CO_2 in the control bottles, then expressed either as the total amount of CO_2 produced within the bottle or as the percentage of organic carbon converted to CO_2.

Results of Experiment No. 1. The mineralization studies showed that the NAS- and TEX-degrading enrichment culture was capable of mineralizing components within both the NAS and TEX mixtures. The percentage of organic carbon converted to CO_2 by the NAS-degrading culture was 48% (day 24) in the NAS bottles and 20% (day 20) in the TEX bottles. The percentage of organic carbon converted to CO_2 by the TEX-degrading culture was 34% (day 30) for the TEX bottles and 20% (day 25) for the NAS bottles.

Experiment No. 2. Mineralization of the four model naphthenic acid compounds was measured as the amount of CO_2 evolved from incubating solutions of the compounds dissolved in nutrient medium and inoculated with enrichment cultures of NAS-degrading microorganisms, TEX-degraders, or oil sands tailings pond water (TPW). Fifteen milliliters of 1 mM solutions of the compounds dissolved in mineral salts medium were placed in 60-ml serum bottles and inoculated (1% v/v) with the different sources of microbes then sealed with robber stoppers. Bottles were incubated at room temperature on a gyratory shaker (100 rpm). After 3, 6, 12, and 24 days, duplicate bottles were acidified and headspace CO_2 determined by GC. The level of CO_2 production was corrected for the amount of CO_2 within the control bottles and expressed as the percentage of organic substrate converted to CO_2.

Results of Experiment No. 2. The following results were obtained:.

Mineralization by day 24, % organic C converted to CO_2:

Substrate	NAS-degraders	TEX-degraders	TPW
CCA	41	56	57
CPA	45	57	58
2MCCA	47	7	67
4PCCA	6	24	24

Experiment No. 3. A 1% (v/v) inoculum of the NAS-degrading enrichment culture was placed in 125-ml Erlenmeyer flasks containing 50 ml of either NAS (30 mg/l) or TEX (1:50 dilution) in mineral salts medium. Control flasks received inoculum of heat-killed cells. The flasks were incubated at room temperature on a gyratory shaker (100 rpm). After an incubation period of 4, 8, and 16 days for NAS and 6, 12, and 24 days for TEX, the contents of two flasks and two control flasks were extracted for GC analysis. Samples were extracted and the carboxylic acids were derivatized to methyl esters prior to analysis.

Derivatized extracts were analyzed by GC with a capillary column and flame ionization detector.

Results of Experiment No. 3. Chromatographic analysis of solution from the control flasks revealed an unresolved series of many overlapping peaks that created a hump in the GC profile. When the mixture that was inoculated with NAS-enrichment culture, a reduction in the size of the hump was evident within 4 days, indicating that components within the naphthenic acid mixture were being degraded. Chromatographic analysis of the TEX samples revealed a similar hump of many overlapping peaks that appeared in the NAS GC profile. Biodegradation of TEX by the NAS-degrading culture did not result in a noticeable reduction in the size of the hump associated with TEX, despite evidence of mineralization of components within the mixture.

Experiment No. 4. A measurement of CO_2 production and O_2 utilization within sealed microcosms was used to monitor microbial activity in samples of TPW, and to determine the effect of nutrient addition (N and P) or carbon substrate addition (cyclohexane pentanoic acid (CPA), sodium salts of naphthenic acids (NAS), or tailings pond extracts of carboxylic acids (TEX)) on the level of microbial activity within TPW.

60 ml of TPW was placed into sterile 125-ml Erlenmeyer flasks, sealed with rubber stoppers in which a sampling port had been drilled and then sealed with clear silicone. Nutrients in the form of N and P were added. Carbon substrates (CPA, NAS or TEX) were added as a filter-sterilized solution to crate a final concentration of 60 mg organic carbon/l. All flasks were incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 80day intervals, 0.5 ml of headspace was sampled and analyzed for CO_2 and O_2 using GC. Following 5 weeks of incubation, the contents of the flasks containing CPA were extracted and analyzed using the procedure described for the GC analysis in experiment 3.

Results of Experiment No. 4. The addition of CPA to TPW resulted in increased microbial activity, as indicated by greater levels of CO_2 production and O_2 utilization when compared with TPW alone. Sterilized TPW demonstrated no CO_2 production or O_2 utilization. Even greater levels of microbial activity were evident when N and P were added in addition to CPA, indicating that mineralization could be enhanced by the addition of mineral nutrients. GC analysis of CPA in TPW microcosms after 35 d of incubation revealed that the concentration of CPA was below the level of detection in 2/3 microcosms and reduced 10-fold in the third microcosm. There was no detectable CPA in the three N and P-amended microcosms.

Similarly, NAS and TEX additions to microcosms increased microbial activity in TPW, although microbial activity was enhanced by the addition of N and P. Increases in both CO_2 evolution and O_2 utilization were seen.

Conclusions. This investigation showed that naphthenic acids, either as a commercial preparation of sodium salt (NAS) or natural extracts from oil sands tailing water (TEX) are capable of being utilized by natural assemblages of microorganisms. Addition of nitrogen and

	phosphorus enhances the utilization of these substrates by the microbes.
Source:	Herman, D.C., P.M. Fedorak, M.D. MacKinnon, and J.W. Costerton. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oils sands tailings.
Reliability:	(2) Reliable with restrictions. The report was a well-documented study that meets basic scientific principles.

4. Ecotoxicity

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Test Substance:	Naphthenic acids
Method/Guideline:	Hart, et al. 1945; Doudoroff et al. 1951
Year (guideline):	N/A
Type (test type):	Static
GLP:	No
Year (study performed):	1965
Species:	zebra fish (*Brachydanio rerio*)
Analytical Monitoring:	No
Exposure Period:	96 hours
Statistical Method: (FT - ME)	Graphical interpolation for determining the LC50.
Test Conditions: (FT - TC)	Test containers were 2.5 gallon aquariums, each fitted with an air stone through which compressed air was bubbled to maintain a 5-9 ppm dissolved oxygen concentration in the dilution water. The aquariums were maintained at a temperature of 24 +/- 1 °C. Dilution water was synthetic soft water prepared with distilled water and ACS grade chemicals.
• **Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.**	The lot of test fish displayed no visible disease. The average size was 3.2 cm total length. Before testing the fish were acclimated to the dilution water for 5 days. During the acclimation period they were fed *Daphnia* and white worms, but were not fed for 36 hours before or during the testing. Test concentrations were prepared by direct addition of the test substance to the test chambers followed by mixing. Test concentrations were control, 7.5, 8.7, 10, 11.5, 13.5, 15.5, 18.0, 21.0, and 24.0 ppm naphthenic acids. After the test solutions were prepared, ten fish were placed in each test container. Controls were run in duplicate, while test levels were run singly. Mortality was evaluated at 24, 48, and 96 hours, and the criteria for death was a cessation of gill movement and failure to respond to mechanical stimulus. Following the 96 hour test period the TLm (median tolerance limit) was determined from visual observation of the dose-response pattern. Where no exact TLm response resulted, the TLm was interpolated from a plot of the concentration and survival data on semi-log paper.
Results: (FT - RS)	96-hour TLm = 16.3 ppm
Units/Value:	

The following dose-response data were provided:

Concentration of Naphthenic acids, ppm	Number Tested	% Dead at 96 hours
0 (control #1)	10	0
0 (control #2)	10	0
7.5	10	0
8.7	10	40

10	10	20
11.5	10	0
13.5	10	20
15.5	10	30
18	10	80
21	10	100
24	10	100

- **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.** The article reported that pH and dissolved oxygen concentrations were taken during the test, but these data were not reported.

Conclusion: (FT - CL)

Reliability: (FT - RL) (2) Reliable with restrictions. The test was conducted under referenced test conditions current for the period in which the study was run. The report provided sufficient details for assessment.

Source: (FT - RE) Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios Brachydanio rerio (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish Lepomis macrochirus Raf. Notulae Naturae. No. 381:1-9.

Hart, W.B., P. Doudoroff, and J. Greenbank. 1945. The evaluation of the toxicity of the industrial wastes, chemicals and other substances to freshwater fishes – The Atlantic Refining Company, Philadelphia, PA. 315 pp.

Dourdoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galstoff, W.B. Hart, T. Patrick, E.R. Strong, E.W. Surber, and W.M. VanHorn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sew. and Ind. Wastes. 23(11):1380-1397.

Other (source): (FT - SO)

FT - Freetext
ME - Method
TC - Test Conditions
RS - Results
CL - Conclusion
RL - Reliability
RE - Reference
SO - Source

Test Substance: Naphthenic acid mixture (commercially available from Eastman Chemicals)

Method/Guideline: Peltier and Weber 1985

Year (guideline): 1985

Type (test type): static acute

GLP: not stated

Year (study performed):

Species: three-spine stickleback (*Gasterosteus aculeatus*)

Analytical Monitoring: no

Exposure Period: 96 hours

Statistical Method: (FT - ME)

Test Conditions: (FT - TC)

- **Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.**

Summary of Test Conditions

Organism age:	juvenile
Test Temperature:	20 °C +/- 2 °C
Photoperiod:	16 h light/8 h dark
Light intensity:	10 – 50 micro-einsteins
Light quality:	wide spectrum fluorescent
Test container:	5 gallon aquaria
Dilution water:	Carquinex Strait
Test Volume:	15 liters
Animals per container:	10
Replicate containers:	2
Number of concentrations:	6 (5 concentrations and a control)
Food:	none
Test duration:	96 h
Test endpoint:	mortality
Salinity	15 parts per thousand
Test pH:	ambient
Test article:	Martinez Refinery effluent (non-toxic) with added naphthenic acids

Test solutions were prepared by creating a 1 percent solution using non-toxic effluent pH adjusted to 12 with sodium hydroxide. The stock solution was mixed overnight prior to use. The stock solution was used to spike non-toxic treated effluent to nominal naphthenic acid concentrations from 2.5 to 30 mg/l.

Test organisms were held at least seven days prior to testing in dilution water. During testing at 24-h intervals, the salinity, temperature, pH, and dissolved oxygen were measured in all control and test tanks. Survival data were taken at 24-h intervals and dead individuals were removed when observed.

Results: (FT - RS)
Units/Value: LC50 estimated to be in the range of 5 mg/l.

The following dose response data were reported:

Concentration (mg/l)	% Survival
0 (control)	100
2.5	60
5	10
10	0
15	0
30	0

- **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.**

Although an LC50 could have been calculated using contemporary methods, the author elected to estimate its value. The report stated that water chemistry data were collected but no data were reported.

Conclusion: (FT - CL)

Reliability: (FT - RL) (2) Reliable with restrictions. A statistically-defined LC50 was not calculated. Water chemistry data were not reported.

Source: (FT - RE) Dorn, P.B. 1992. Case Histories – The petroleum refining industry. In: Ford, D.L. (ed.). Water Quality Management Library, Volume 3, Toxicity Reduction Evaluation

and Control. Technomic Publishing Co., Lancaster, PA. pp 183 – 223.

Peltier, W.H., and C.I. Weber, eds. 1985. Method for measuring acute toxicity of effluents to freshwater and marine organisms, 3rd edition. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/4-85-014. 230 pp.

Stephan, C.E. 1977. Method for calculating an LC50. In: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA. pp 65-84.

Other (source): (FT - SO)

FT - Freetext
ME - Method
TC - Test Conditions
RS - Results
CL - Conclusion
RL - Reliability
RE - Reference
SO - Source

4.9 ADDITIONAL REMARKS

Memo: Effect of naphthenic acids on survival of zebra fish (*Brachydanio rerio*) embryos

Remark: Zebra fish embryos were exposed for 48 hours to a range of naphthenic acids concentrations to determine the TLm (median tolerance limit) for embryo survival. Embryos were collected from a culture unit once they attained Stage 21 as designated by Hisaoka and Battle (1958). Ten embryos were exposed to each test solution and control in petri dishes holding 45 ml of the exposure solutions. Exposure solutions were prepared by diluting a stock solution of naphthenic acids (100 mg naphthenic acids in 50 ml acetone) with water. In addition to a control group, nine concentrations of naphthenic acids were prepared at 2.4, 3.2, 4.2, 6.5, 10, 15.5, 24, 32, and 42 ppm naphthenic acids. Mortality was assessed at 24 and 48 hours of exposure. The embryo was considered dead if it had an opaque appearance.

A TLm of 3.5 ppm was obtained by plotting the survival versus concentration on semilog paper and interpolating the 50% survival concentration. The following dose response was given:

Test Concentration, ppm	Percent Dead
0 (control)	0
2.4	0
3.2	40
4.2	70
6.5	100
10	100
15.5	100
24	100
32	100
42	100

Source: Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios Brachydanio rerio (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish Lepomis macrochirus Raf. Notulae Naturae. No. 381:1-9.

Hisaoka, K.K., and H.I. Battle. 1958. The normal development stages of the zebra-fish, *Brachydanio rerio* (Hamilton-Buchanan). J. Morph. 102(2):311-327.

Reliability: (2) Reliable with restrictions. Although the test was conducted prior to the time of standardized test methods, the report provided sufficient information on the dose-response pattern for the test substance.

Memo: Effect of naphthenic acids on survival of bluegill (*Lepomis macrochirus*)

Value: 48-hour TLm = 5.6 mg/l naphthenic acids

Remark: The value was reported in a summarized journal article (Cairns et al., 1965) as originating in Cairns and Scheier (1962).

Source: Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios Brachydanio rerio (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish Lepomis macrochirus Raf. Notulae Naturae. No. 381:1-9. Acad. Nat. Sci. Philadelphia.

Cairns, J. Jr., and A. Scheier. 1962. The effect of temperature and hardness of water upon the toxicity of naphthenic acids to the common bluegill (Lepomis macrochirus Raf.) and the pond snail (Physa heterostropha Say). Notulae Naturae. No. 353: 111 pp. Acad. Nat. Sci. Philadelphia.

Reliability: (3) Not reliable. The endpoint was cited in the text of a journal article without details of the test.

Memo: Effect of naphthenic acids on survival of bluegill (*Lepomis macrochirus*)

Value: 96-hour LC50 = 0.0026 mg/l

Remark: Test chambers were 30x60x30 cm all-glass vessels. Dilution water was well water. Testing was performed at a temperature of 22 +/- 1°C under a 16-h light/8-h dark photoperiod.

The test included five concentrations of the test substance and a dilution water control. Each test level included 20 fish distributed 10 each to two replicate chambers per treatment.

Dissolved oxygen ranged from 4.3 to 8.1 mg/l, pH ranged from 7.4 to 8.0, and temperature ranged from 22 to 24 °C when measured daily during the test. Specific conductance between the test solutions remained constant at 550 (no units given) when measured at the beginning of the test.

The report stated that serial dilutions of the test product were created for testing, although no details were given as to how the serial dilutions or the original solution was created. The raw data indicated that concentrations were expressed as a percent, while the LC50 and confidence interval was reported as parts per million. There was no explanation how the values for percent were related to parts per million.

Critical details of testing procedures and animal culture were omitted from the report.

Source: Exxon Corporation. 1980. Aquatic bioassay testing of Exxon Corporation's experimental compounds (MRD 78-100). Report by Battelle Columbus Laboratories, Columbus, Ohio.

5. Acute Toxicity

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_{50}
Value:	5.88 (4.31-8.02) g/kg bw
Species:	Rat
Strain:	Wistar
Sex:	Male
Number of Animals:	5 per dose level (7 dose levels)
Vehicle:	None – administered undiluted
Year:	1979
GLP:	Unable to determine
Test Substance:	MRD-79-10 (Raw naphthenic acid derived from kerosene)
Method	Seven groups of 5 male rats were dosed at 1.0, 1.47, 2.15, 3.16, 4.64, 6.81, and 10 g/kg of body weights. Food and water were freely available except for the 16-20 hours prior to dosing. The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.
Result:	Deaths occurred at the four highest dose levels: 3.26, 4.64, 6.81, and 10 g/kg bw. 8/10 animals died at the two highest dose levels. Significant predeath toxic signs included tremors, lethargy, ptosis, ataxia, prostration, negative righting reflex, flaccid muscle tone, piloerection, diarrhea, chromodacryorrhea, dyspnea and chromorhinorrhea. Body weight changes were noted in the survivors. Significant necropsy findings in the animals that died during the study included dilated hearts and gastrointestinal irregularities. The LD_{50} was determined to be 5.88 (4.31-8.02) g/kg bw
Reliability:	(1) Reliable without restrictions; appears to be comparable to a guideline study with adequate experimental details provided; although the investigators used male rats only, there is sufficient experimental detail to make a conclusion on the study's validity, and the results can be used to assess the potential acute toxicity of naphthenic acid.

Source	Exxon, Acute Oral Toxicity of MRD-79-10 in Rats, MB 79-3702, 1979.
Type:	LD_{50}
Value:	3.0 g/kg bw (fraction from crude kerosene acids) 5.2 g/kg bw (fraction from mixed crude oils)
Species:	Rat
Strain:	No information
Sex:	No information available
Number of Animals:	"Sufficient animals ...so the the LD50 dose could be computed by either the Weil or the Litchfield and Wilcoxon method"
Vehicle:	None -- administered undiluted
Year:	1955
GLP:	Unable to determine
Test Substance:	1) 7-93% Naphthenic acid fraction from crude kerosene acids 2) 65-69% Naphthenic acid fraction from mixed crude oils
Method	"The LD50 ..was determined in rats by use of screening test procedures similar to those of Smyth and Carpenter." (Smyth, H.F., and C.P. Carpenter. 1944. Place of the range finding test in the industrial toxicology laboratory. J. Indust. Hyg. & Tox. 26: 269.
Result:	Death appears to result from gastrointestinal disturbances, with the mortality peak occurring on the third to fourth day after administration. The animals exhibited anorexia, inanition, diarrhea, and asthenia. The LD_{50}s were determined to be 3.0 g/kg bw (fraction from crude kerosene acids) and 5.2 g/kg bw (fraction from mixed crude oils)
Reliability:	(2) Reliable with restrictions; Although not a guideline or GLP study, and some of the experimental details are not available, the study does appear to be well-conducted, and cites that the investigators followed published methodologies for conducting a statistically valid LD50. The data are supportive of other acute toxicity studies reported by Exxon and Pennisi.
Source	Rockhold, W.T. 1955. The toxicity of naphthenic acids and their metal salts. Archs Ind Hlth 12, 477-482.

Type:	LD_{50}
Value:	3550 mg/kg bw
Species:	Mice
Strain:	White – no other information
Sex:	Male
Number of Animals:	No information available
Vehicle:	No information available
Year:	1977
GLP:	Unlikely
Test Substance:	Naphthenic Acid – no further description
Method	Not described
Result:	Oral administration resulted in 1) CNS depression without analgesia and no loss of corneal reflex, 2) corneal eye opacity, 3) dryness of mouth, 4) convulsions, 5) diarrhea, and 6) death due to respiratory arrest.
Reliability:	(4) Not assignable. This information is taken from a published, meeting abstract. The level of experimental details provided is not sufficient to verify the conclusions.
Source	Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181.

Type:	Acute Oral Toxicity Study (Not LD50)
Value:	Not applicable
Species:	Rat
Strain:	Wistar
Sex:	Male/Females
Number of Animals:	10 Females/dose (3 doses, plus control) 10 Males/dose (1 dose, plus control)
Vehicle:	Aqueous solutions of naphthenic acids/Water
Year:	2002

GLP:	Unable to determine
Test Substance:	Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 55,080, 5508 or 550.0 mg/l naphthenic acids – derived from athabasca sands sands tailings.
Method	Female rats were given a single oral dose of naphthenic acids at 3, 30 or 300 mg/kg bw, while male rats received 300 mg/kg. Control animals were given tap water. All animals were monitored continuously for 12 hr after dosing, and thereafter daily. Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination. Statistical analysis was performed by using a one-way ANOVA to compare means of female dose and control groups with respect to consumption, body weights, and organ weights. A pair wise multiple comparison test was then used in cases where statistical significance was reached. For the male dose and control groups, a Student's t-test was used to compare group means. Probability values of $p \leq 0.05$ was considered statistically significant.
Result:	The following effects were seen in the high dose groups: • Decreased food consumption immediately following dosing. • Lethargy and mild ataxia (2/10 females, 3/10 males) • Statistically significant increase relative organ weights: ovaries, spleen in females- testes, heart in males • 7/10 females and 6/10 males exhibiting eosinophilic pericholangitis • 6/10 males and 2/10 females with brain hemorrhage. The following effects were seen in the mid dose group: • 7/10 females and 4/10 males with heart lesions.
Reliability:	(2) Reliable with restriction. The study is not an acute toxicity study as defined by OECD SIDS/HPV, however it appears to be well conducted and provides additional information regarding potential acute, non-lethal effects of naphthenic acids following oral exposure.
Source	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.

5.1.2 ACUTE DERMAL TOXICITY (WITH IRRITATION)

Type:	LD_{50}
Value:	> 3.16 g/kg bw
Species:	Rabbit

Strain:	NZ White
Sex:	Male/Female
Number of Animals:	2 per sex
Vehicle:	None – administered undiluted
Year:	1979
GLP:	Unable to determine
Test Substance:	MRD-79-10 (Raw naphthenic acid derived from kerosene)
Method	3.16 g/kg naphthenic acid was applied dermally to the clipped abraded abdomens of each animal. The area was covered with gauze and secured by a thick plastic binder, which was removed after 24 hours, and the skin washed with water or corn oil. According to experimental protocol, no deaths occurred at the initial level, no addition animals were dosed. If one animal died, the experiment was to be repeated using 3 more groups of animals dosed at varying levels. Following the skin wash, animals were observed for mortality and toxic effects at 2 hr and 4 hr, and once daily thereafter. Body weights were recorded pretest and at termination. Dermal irritation was recorded at 24 hr, 3, 7, 10 and 14 days. The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.
Result:	No deaths occurred at the 3.16 mg/kg dose level. Most of the animals (3/4) appeared normal during the first 2 to 4 hours of dosing, after which symptoms of toxicity were observed. 3 out of 4 animals (1 male, 2 female) showed signs of toxicity until day 12 or 13. During the first 5 days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The LD_{50} was determined to be greater than 3.16 g/kg bw Redness and irritation scores were recorded at 24 hr, 3, 7, 10 and 14 days post-washing. 4 Hour occluded sites (DOT, OECD methods) Mean values (24, 48 & 72 hours) for erythema and edema at the intact sites were 1.69 and 1.3 respectively. The initial response of the skin to the test material was slight, with little difference in response between intact or abraded sites.

The material was judged to be moderately to severely irritating to the occluded skin.

Actual scores were:

Erythema/Eschar Scores

Animal Number	1 day	3 day	7 day	10 day	14 day
1M	2	2	4	4	1
2M	1	2	4	4	1
3F	2	4	4	4	0
4F	2	3	4	4	0
Note: All animals showed signs of scar formation after 14 days.					

Edema

Animal Number	1 day	3 day	7 day	10 day	14 day
1M	3	2	2	2	1
2M	2	3	2	2	0
3F	3	3	2	2	0
4F	3	3	2	2	0

Reliability: (1) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.

Source Exxon, Acute Dermal Toxicity of MRD-79-10 in Rabbits, MB 79-3702, 1979.

5.2.1 EYE IRRITATION

Type: EYE IRRITATION

Species: Rabbit

Strain: NZ White

Sex: Male/Female

Number of Animals: 3 per sex

Concentration :	None – administered undiluted
Year:	1979
GLP:	Unable to determine
Test Substance:	MRD-79-10 (Raw naphthenic acid derived from kerosene)
Method	0.1 ml naphthenic acid was placed into the conjunctival sac of eye of each of the six rabbits. The lids were held together briefly to insure adequate distribution. The untreated eye served as a control. The rabbits were observed at 1 and 4 hours, and on days 1, 2, 3, 4, and day 7. If a positive score (any score for iritis or opacity, or a score of 2 or more for redness or chemosis) was noted on day 7, ocular reactions were scored on day 10. Likewise readings on day 14 were given if there was a positive reaction on day 10. Fluorescein was used in examining ocular reactions on day 3 and after. The Draize technique was used as the scoring system.
Result:	The following is a summary of data taken from the report: One animal had a positive corneal score that was noted on days 1 and 2. One animal had a positive iris score which was noted during hours 1 and 4. All animals exhibited positive conjunctival scores at some pint during the first three days of observation. By day 4, no animals showed positive scores. abraded sites. The material was judged to be an irritant. (According to Draize chart, 4 to 6 rabbits with positive scores observed at 24, 48 or 72 hours). In a later Exxon summary report, eye irritation was judged to be moderate (Exxon, 1980).
Reliability:	(1) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.
Source	Exxon, Eye Irritation Study of MRD-79-10 in Rats, MB 79-3702, 1979.

5.4 REPEATED DOSE TOXICITY

Type:	Subchronic (90 Day)
Species:	Rat
Sex:	Females
Strain:	Wistar
Route of administration:	Oral

Exposure period:	90 days
Frequency of treatment:	1 dose/day (Mon. – Fri, 5 days/week)
Doses/No. of animals:	0.6, 6 or 60 mg/kg bw (aqueous solutions of naphthenic acids); 12 animals per dose level
Control group:	Water – 7.0 ml tap water
Year:	2002
GLP:	Unable to determine
Test Substance:	Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 8549, 845.9 or 84.50 mg/l naphthenic acids derived from Athabasca sands sands tailings.
Method:	Female rats were administered naphthenic acid (orally) at doses of 0.6, 6, or 60 mg/kg/day, 5 days per week for 90 days. Control animals were given 7 ml tap water. All animals were monitored daily . Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Blood samples were collected from the ventral tail vein on day 45 of dosing and analyzed for plasma biochemical and hematological effects. Similarly, blood samples taken via cardiac puncture on day 91 were analyzed. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination. Statistical analysis was performed by using a one-way ANOVA to compare group means for consumption, plasma biochemical/ hematological parameters , and organ weights, while a one-way repeated measure ANOVA was used to compare body weight trends. Probability values of $p \leq 0.05$ was considered statistically significant.
Result:	The following significant effects were seen in the high dose groups: • Decreased food consumption immediately following dosing. • Severe, clonic seizures lasting 20 sec (25%) of animals, observed after day 40 – after which all animals, except one that died, resumed normal activity.* • Lower mean body weight throughout the exposure period. • Increased relative organ weights: liver, kidney and brain • Reduction in plasma cholesterol on days 45 and 91 (41 and 43%), Increase in amylase activity on day 45 and 91 (33 and 30%) • Less pronounced differences in total protein concentration (increased) and albumin/globulin ratio (decreased) • 5/12 rats with increased glycogen storage. The following effects were seen in the mid-dose group: • Severe, clonic seizures lasting 20 sec (17%) of animals, observed after day 40 – after which all animals except one that died, resumed normal activity.* • 3/12 rats with increased glycogen accumulation The following effects were seen in the low-dose group:

	• 2/12 rats with increased glycogen accumulation *Note: Rats in the low-dose (8%) and control (17%) demonstrated milder episodes, characterized primarily by muscle twitching. Dose-related changes in liver tissue with respect to glycogen accumulation.
Reliability	(2) Reliable with restriction. The study is not a typical subchronic toxicity study as defined by OECD SIDS/HPV, i.e., the study was conducted with female rats only and examined a limited number of organs. However, it is well-conducted and provides limited information regarding potential subchronic effects of naphthenic acids following oral exposure.
Source:	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.
Type:	Subchronic (30 Day)
Species:	Mice
Sex:	Male
Strain:	Wistar
Route of administration:	Oral
Exposure period:	30days
Frequency of treatment:	Daily
Doses/No. of animals:	1000 mg/kg bw (no information on number of animals per dose)
Control group:	No information available
Year:	1977
GLP:	Unlikely
Test Substance:	Naphthenic acid – no further information.
Method:	Male rats were given daily oral doses of 1000 mg/kg naphthenic acids. No other experimental details provided in abstract.
Result:	The following statements appeared in the abstract: Repeated daily administration (30 days) of naphthenic acid at doses of 1000 mg/kg orally .. revealed a few cases of (1) CNS depression without analgesia and no loss of the corneal reflex (2) hematological changes, (3) weight loss leading eventually to death due to respiratory arrest, (4) gross morphological changes in the liver and stomach, and (5) histomorphological changes in a few selected organs.
Reliability	(4) Not assignable. This information is taken from an abstract. The protocol of the study does not appear to be comparable to a guideline

study, and the level of detail is insufficient to judge its validity.

Source:

Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181. [meeting abstract]

5.5 GENETIC TOXICITY IN VITRO

The following salts of naphthenic acid were tested using National Toxicology Program protocols and conducted in accordance with GLP's. Consequently they have ratings of (1), reliable without restriction:

	Calcium Naphthenate	Sodium Naphthenate
Salmonella Mutagenicity Test	Negative	Negative
Chromosome Aberration Test	---	Negative
Sister Chromatid Exchange Test	---	Positive

Source: NTP. 2003. http://ntp-server.niehs.nih.gov/htdocs/Overviews/GenProtocolsPg.html.

5.6 GENETIC TOXICITY IN VIVO

No data available.

5.7 CARCINOGENICITY

Species:	Mice
Sex:	Female
Strain:	No information available
Route of administration:	Dermal
Exposure period:	2 yr
Frequency of treatment:	2 times/day
Doses/No. of animals:	0.05 ml neat - 50 animals
Control group:	No information available
Year:	1987
GLP:	Unknown
Test Substance:	Calcium naphthenate
Method:	Not described; listed in summary as "non-TSCA Protocol/Guideline (voluntary test)"
Result:	The following statements appeared in the abstract: Clinical observations included mild irritation, hair loss, shiny patches on the skin, and flaking skin surfaces. These progressed to moderate irritation (observed with sores and scabs on the treated site), or severe irritation caused by large sores or visible ulcers. In the negative control group, no cutaneous tumors developed at or distant to treated sites. Twelve epidermal and one dermal tumor at the treated sites were observed in eight mice that were exposed to the test material. Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present.
Reliability	(4) Not assignable. This information is taken from an EPA site that summarizes results of testing submitted under TSCA. The protocol of the study does not appear to be comparable a guideline study as indicated in the summary.
Source:	U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). http://www.epa.gov/opptintr/chemtest/naphthst.htm.

5.8 EFFECTS ON REPRODUCTION

Type:	One Generation Reproduction
Species:	Rabbit
Sex:	Male (10)/Female (2)
Strain:	No information available
Route of administration:	Dermal
Frequency of treatment:	6 hr/day, 5 d/wk, 10 weeks
Doses/No. of animals:	2 ml (neat) – 10 male (2 female animals not treated)
Control group:	No information available
Method:	10 week exposure of males prior to mating
Year:	1984
GLP:	Unknown
Test substance:	Calcium naphthenate
Method:	Not described; listed in summary as "non-TSCA Protocol/Guideline (voluntary test)"
Result:	The following statements appeared in the available summary: There were no systemic toxicity, application site toxicity, or statistically significant changes in body weights observed in the test animals during the 10 week exposure period or the 12 week post-exposure period. In the male animals, there were no significant changes in the testes weights. In the females, there were no significant differences in the number of implantations, or in pre-and post-implantation losses. In addition, there were no differences in viable fetuses to those females that were mated with exposed males compared to those mated with unexposed males. The study also reported that there were no macroscopic or microscopic pathological findings in the male reproductive tract.
Reliability:	(4) Not assignable. This information is taken from an EPA site that summarizes results of testing submitted under TSCA. The protocol of the study does not appear to be comparable a guideline study as indicated in the summary.
Source:	U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). http://www.epa.gov/opptintr/chemtest/naphthst.htm.

5.9 DEVELOPMENTAL TOXICITY

Species:	Rat
Sex:	Female
Strain:	Wistar
Route of administration:	Oral
Dose:	0.6, 6 or 60 mg/kg bw
Exposure period:	"Pre-breeding, breeding and gestation" - no other details provided
Frequency of treatment:	Daily
Year:	2002
GLP:	Unknown
Test Substance:	Naphthenic acid isolated from Athabasca oil sands tailings.
Method:	Oral doses of 60 mg/kg/day were given to female rats during pre-breeding, breeding and gestation.
Result:	The following description was given: Reproductive toxicity testing demonstrated dramatic effects on female fertility at an oral dosage of 60 mg/kg/day during pre-breeding, breeding and gestation. While control and low dose (6 mg/kg/day) animals achieved 93 and 100% reproductive success, respectively, only 7% of females dosed at 60 mg/kg/d successfully bore a litter. Total cholesterol of the latter group was 30% lower than controls. Mating and ovulation were comparable amongst control and dose groups, while fetal malformations were not apparent in any offspring. Results suggest that the dose-related infertility may be associated with poor embryonic implantation, an effect that might be secondary to depressed sex hormone production requiring cholesterol as a precursor.
Reliability:	(4) Not assignable. This information is taken from an abstract. The protocol of the study does not appear to be comparable to a guideline study, and the level of detail is insufficient to judge. However, it may be useful in establishing dose levels for a more in-depth study.
Source:	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002b. Mammalian toxicity of naphthenic acids derived from the Athabasca Oil Sands (AOS). Toxicologist 66(1-S): 64-5. [meeting abstract]

1. General Information

ID 1338-24-5

Date December 22, 2005

1.0 SUBSTANCE INFORMATION

Generic Name	:	
Chemical Name	:	Naphthenic acids
CAS Registry No.	:	1338-24-5
Component CAS Nos.	:	
EINECS No.	:	215-662-8
Structural Formula	:	
Additional description	:	Naphthenic acid is mixture of various carboxylic acids which occur naturally in crude petroleum. The most common class of acid is derived from cyclopentane and has the general formula CnH2n-202, where n = 8 to 12. This basic cyclopentane structure can be more or less highly alkylated. Other classes of acids include simple paraffinic acids of the general formula CnH2n02 where n = 5 to 8, and acids with larger more complicated molecules of the general formula CnH2-402, where n = 13 to 23. The classes and proportions of individual naphthenic acids in the overall mix vary according to the origin of the crude oil.
Molecular Weight	:	Generally between 140 and 450
Synonyms and Tradenames	:	
References	:	AGS Chemicals Ltd., 2003, Product Information, Naphthenic Acid, (); Headley, J.V. and D.W. McMartin, 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments, Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances & Environmental Engineering, A39(8):1989 -2010.

2.1 MELTING POINT

Type	:	
Guideline/method	:	
Value	:	-35 to +2°C
Decomposition	:	at °C
Sublimation	:	
Year	:	
GLP	:	
Test substance	:	Commercially available naphthenic acid
Method	:	
Method detail	:	
Result	:	
Remark	:	A range of melting points would be expected, based upon the hydrocarbon composition of the specific naphthenic acid mixture. Estimated melting points were calculated for one to four ring cycloalkyl naphthenic acid structures with molecular weights ranging from 260 to 320; these dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands. Melting points calculated using EPIWIN v3.10 ranged from 117ºC to 160ºC for these structures (Appendix C). In contrast, structural profiles of commercial naphthenic acids have been shown to differ substantially from natural extracts (Rogers et al., 2002, cited in Appendix C). Product literature for commercially available naphthenic acid provides a melting point range of -35º to +2ºC (AGS Chemicals Ltd., 2005).
Reliability	:	[4] Not assignable. Original source data (for commercially available naphthenic acid) were not available for review.
Reference	:	AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid (); API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C).

2.2 BOILING POINT

Type	:	
Guideline/method	:	
Value	:	140ºC to 200ºC
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	Commercially available naphthenic acid
Method	:	
Method detail	:	
Result	:	
Remark	:	A range of boiling points would be expected, based upon the hydrocarbon composition of the specific naphthenic acid mixture. Estimated boiling points were calculated for one to four ring cycloalkyl naphthenic acid structures with molecular weights ranging from 260 to 320; these dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands. Boiling points calculated using EPIWIN v3.10 ranged from 233ºC to 375ºC for these structures (Appendix C). In contrast, structural profiles of commercial naphthenic acids have been shown to differ substantially from natural extracts (Rogers et al., 2002, cited in Appendix C). Product literature for commercially available naphthenic acid provides a boiling point range of 140º to 200ºC (AGS Chemicals Ltd., 2005).
Reliability	:	[4] Not assignable. Original source data (for commercially available naphthenic acid) were not available for review.
Reference	:	AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid (); API,

2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C).

2.3 DENSITY

Type	:	
Guideline/method	:	
Value	:	0.91 to 0.96 g/cm^3 at 15ºC
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	[4] Not assignable. Original source data were not available for review.
Reference		AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid (http://www.ags-chemicals.com)

2.4 VAPOR PRESSURE

Type	:	
Guideline/method	:	
Value	:	
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	It was estimated using EPIWIN v.310 that the vapor pressures of the components of naphthenic acid mixtures would be near or below the measurable limits cited in standard guideline methods and thus, the total vapor pressure of naphthenic acids is expected to be exceedingly low (Appendix C).
Reliability	:	[2] Reliable with restrictions, as assessed in Appendix C.
Reference	:	API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C).

2.5 PARTITION COEFFICIENT

Type	:	
Guideline/method	:	
Partition coefficient	:	
Log Pow	:	
pH value	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Using EPIWIN v3.10, partition coefficients were estimated for a range of molecular weight naphthenic acids spanning the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts. Resulting log Kow values ranged from 5.1 to 9.2. Mixtures

		of naphthenic acids with a significant proportion of structures with molecular weights below 250 will likely show lower log Kow values than those presented. (Appendix C).
Reliability	:	[2] Reliable with restrictions, as assessed in Appendix C.
Reference	:	API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C).

2.6.1 SOLUBILITY IN WATER

Type	:	
Guideline/method	:	
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at °C
Description	:	
Stable	:	
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	
Deg. products CAS#	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Using EPIWIN v3.10, water solubility was estimated for a range of molecular weight naphthenic acids spanning the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts. Resulting water solubility estimates ranged from 0.0003 to 2.1 mg/L. Mixtures of naphthenic acids with a significant proportion of structures with molecular weights below 250 will likely show greater water solubilities than those presented. (Appendix C)
Reliability	:	[2] Reliable with restrictions, as assessed in Appendix C.
Reference	:	API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C).

2.7 FLASH POINT

Type	:	
Guideline/method	:	
Value	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	.

3.1.1 PHOTODEGRADATION

Type		
Guideline/method	:	
Light source	:	
Light spectrum	:	
Relative intensity	:	based on
Spectrum of substance	:	lambda (max, >295nm) :
		epsilon (max) :
		epsilon (295) :
Conc. of substance	:	at °C
DIRECT PHOTOLYSIS		
Halflife (t1/2)	:	
Degradation	:	% after
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	
Degradation	:	
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	Three naphthenic acid mixtures (two commercially-available and one extracted from an Athabasca Oil Sands tailings pond) as well as three individual naphthenic acids: 4-methylcyclohexaneacetic acid (4-MCHAA), 4-methylcyclohexanecarboxylic acid (4-MCHCA), and 3-methylcyclohexane-carboxylic acid (3-MCHCA).
Deg. products CAS#	:	
Method	:	
Method detail	:	Experiments were conducted with natural sunlight, artificial solar radiation in growth chambers using an incandescent and fluorescent lamp canopy, artificial UV-range solar radiation in quartz annular photochemical cells, and UV-254 ultraviolet lamps in quartz annular photochemical cells. All aqueous solutions of naphthenic acids were prepared in Athabasca Rivver water and 1 mL aliquots collected at selected time intervals to assess photochemical degradation as well as toxicity changes. Concentrations were 0.5 to 125 mg/L depending upon the compound or mixture under study. Control reactors were monitored simultaneously in the absence of UV light in natural water and in both the absence and presence of UV light in reagent water. The production of hydroxyl radicals during photolysis was measured with a benzoic acid (BA) chemical probe. As BA is lost and 3-hydrobenzoic acid (HBA) formed when the hydroxyl radical is scavenged, the hydroxyl radical concentration is calculated and the primary method of photolysis determined (e.g., indirect or direct). Benzoic acid was added to selected samples at a concentration of 6.4 mg/L. Loss of BA and production of HBA was measured using LC/MS. The concentration of the naphthenic acids was also measured using LC/MS.
Result	:	Naphthenic acid photolysis resulting from exposure to natural and artificial sunlight was limited. After one week of exposure to natural solar radiation, no individual compounds or mixtures were significantly degraded, although compositional changes were noted in the mixtures. Artificial solar radiation was similarly ineffective. Exposure to UV-245 radiation induced the most photolysis, but was only particularly effective on 4-MCHAA (half-life 3.2 – 3.6 hours) and was not an efficient means for complete removal of the other individual acids or complex mixtures from natural waters.

Remark	:	
Reliability	:	[2] Reliable with restrictions. Not a guideline study, but sufficiently documented to provide useful information.
Reference	:	McMartin, D.W., J.V. Headley, D.A. Friesen, K.M. Peru, and J.A. Gillies, 2004. Photolysis of naphthenic acids in natural surface water, Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances & Environmental Engineering, A39(6):1361-1383.

3.1.2 DISSOCIATION

Type	:	
Guideline/method	:	
pKa	:	
Year	:	
GLP	:	
Test substance	:	
Approx. water solubility	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Naphthenic acids exist as weak acids, with most pKa values being reported at about 5. At low pHs, they exist in their undissociated form and tend to partition onto solids. At high pHs, they exist in their dissociated form and become more mobile. (Appendix C)
Reliability	:	
Reference	:	API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C).

3.2.1 MONITORING DATA

Type of measurement	:	
Media	:	
Concentration	:	mg/l
Substance measured	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

3.3.1 TRANSPORT (FUGACITY)

Type	:	
Media	:	
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Year	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Using EPIWIN v3.10, Level I fugacity modeling was performed for a range

of naphthenic acids covering the predominant molecular weight and ring structures reported to predominate in Athabasca oil sands extracts. The principal distribution of these constituents following environmental release would be to soil and/or sediment, with overwhelming (98%) partitioning to soil. (Appendix C).

Reliability : [2] Reliable with restrictions, as assessed in Appendix C.

Reference : API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C).

3.5 BIODEGRADATION

Type :

Guideline/method : Non-guideline study

Inoculum : Sodium naphthenate-degrading enrichment cultures derived from oil sands tailings water.

Concentration : related to
related to

Contact time :

Degradation : (±) % after day(s)

Result :

Kinetic of test subst. : 50% converted to CO_2 in a 24-d period.
%
%
%
%

Control substance :

Kinetic : %
%

Deg. product :

Year : 1994

GLP :

Test substance : Commercial sodium naphthenate mixture

Deg. products CAS# :

Method :

Method detail :

Result : Commercial mixtures of the sodium salts of naphthenic acids were shown to degrade and mineralize to CO_2 when inoculated with microbial populations indigenous to oil sands tailings. Approximately 50% of the organic carbon was converted to CO_2 over a 24-d period. Three of four model naphthenic acid compounds were also degraded by the enrichment cultures, with approximately 40-50% of the organic carbon converted to CO_2 over a 24-d period.

Remark : Additional studies by Clemente et al. (2004) monitored the concentration and composition of naphthenic acids in aerobic biodegradation studies using sodium salts of naphthenic acids. Within 10 days of incubation with enrichment cultures on naphthenic acid-degraders, naphthenic acids concentration dropped from about 100 to <10 mg/L, accompanied by release of about 60% of the carbon as CO_2. GC/MS results indicated that the lower molecular weight acids (n = 5-13) were degraded more readily than high molecular weight acids. (lemente, J.S., M.D. Mackinnon, and P.M. Fedorak, 2004. Aerobic biodegradation of two commercial naphthenic acids preparations, Environ. Sci. Technol. 38:1009 – 1016)

Reliability :

Reference : Herman et al. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can. J. Microbiol. 40:467-477; Appendix C.

3.7 BIOCONCENTRATION

Type	:		
Guideline/method	:		
Species	:		
Exposure period	:	at	°C
Concentration	:		
BCF	:		
Elimination	:		
Year	:		
GLP	:		
Test substance	:		
Method	:		
Method detail	:		
Result	:		
Remark	:		
Reliability	:		
Reference	:		

4.1 ACUTE TOXICITY TO FISH

Type	:	Static acute
Guideline/method	:	
Species	:	Zebra fish (*Brachydanio rerio*)
Exposure period	:	96 hours
NOEC	:	
LC0	:	
LC50	:	16.3 ppm (TLm)
LC100	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	No
Year	:	
GLP	:	No
Test substance	:	Naphthenic acids
Method	:	
Method detail	:	Tests were conducted in 2.5 gallon aquariums, at 24 ± 1ºC, with aeration to maintain dissolved oxygen at 5-9 mg/L. Dilution water was synthetic soft water. Fish average size was 3.2 cm total length. Fish were not fed for 36 hours prior to the test or during the test. Test concentrations were prepared by direct addition of the test substance to the test chambers followed by mixing. Test concentrations were control, 7.5, 8.7, 10, 11.5, 13.5, 15.5, 18.0, 21.0 and 24.0 ppm. Ten fish were placed in each test container. Controls were run in duplicate while test levels were run singly. Mortality was evaluated every 24 hours.
Result	:	The TLm (median tolerance limit) was determined from visual observation of the dose-response pattern, or interpolated from a plot of the concentration and survival data on semi-log paper. The 96-h TLm was 16.3 ppm.
Remark	:	In a related study, the 48-h TLm for zebra fish embryos was reported to be 3.5 mg/L. Another study reported that the 96-LC50 for a commercially-available naphthenic acid mixture for three-spine stickleback (*Gasterosteus aculeatus*) was estimated to be in the range of 5 mg/L (Dorn, P.B., 1992, cited in Appendix C). Other available data indicate a 48-h TLm of 5.6 mg/L for bluegill (*Lepomis macrochirus*) (Cairns et al., cited in Appendix C). Commercial sodium salts of naphthenic acid produced LC50 values of 50 mg/L for kutum (*Rutulis frisii* kutum) and sturgeon (*Acipenser gueldenstaedi*) and 75 mg/L for roach (*Rutulis rutulis* caspicus) (Dokholyan and Magomedov, 1983, cited in Rogers, V.V., et al., Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355).
Reliability	:	[2] Reliable with restrictions, as assessed in Appendix C.
Reference	:	Cairns, J. et al., 1965. A comparison of the sensitivity of certain chemicals of adult zebra danios *Brachydanio rerio* and zebra danio eggs with that of adult bluegill sunfish *Lepomis macrochirus* Raf. Notulae Naturae 381:1-9, as cited in Appendix C.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	
Guideline/method	:	
Species	:	
Exposure period	:	

NOEC	:	
EC0	:	
EC50	:	
EC100	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	A 96-h LC50 of 4.8 mg/L for calcium naphthenate has been reported for the marine copepod, *Nitocra spinipes*. (Bengtsson, B.E. and M. Tarkpea. 1983. The acute aquatic toxicity of some substances carried by ships. Mar. Pollut. Bull. 14:213-214). The zooplankton species *Nephargoides maeoticus* tolerated naphthenic acids concentrations up to only 0.15 mg/L (Dokholyan and Magomedov, 1984, cited in Clemente, J.S. and P.M. Fedorak, 2005, A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids, Chemosphere 60:585-600).
Reliability	:	[4] Not assignable. Secondary reference.
Reference	:	

4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

Type	:	
Guideline/method	:	
Species	:	
Endpoint	:	
Exposure period	:	
NOEC	:	
LOEC	:	
EC0	:	
EC10	:	
EC50	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	The toxicity of naphthenic acids to populations of the freshwater diatom, *Navicula seminulum*, has been measured. The 96-h EC50 for growth ranged from 26.0 – 80.5 mg/L (Academy of Natural Sciences. 1960. Cited in the EPA ECOTOX Database 2005. http://www.epa.gov/ecotox).
Reliability	:	[4] Not assignable. Secondary reference.
Reference	:	

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In vtro/in vivo	:	
Type	:	
Guideline/method	:	
Species	:	
Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Route of administration	:	
Exposure time	:	
Product type guidance	:	
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	1st.: 2nd.: 3rd.:
Toxic behavior	:	
Deg. product	:	
Deg. products CAS#	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

5.1.1 ACUTE ORAL TOXICITY

Type	:	Acute oral LD50
Guideline/Method	:	
Species	:	Rat
Strain	:	Wistar
Sex	:	Male
Number of animals	:	5 per dose level (7 dose levels)
Vehicle	:	None - administered undiluted
Doses	:	1.0, 1.47, 2.15, 3.16, 4.64, 6.81, and 10 g/kg bw
LD50	:	5.88 g/kg bw (4.31 - 8.02 g/kg bw)
Year	:	1979
GLP	:	unknown
Test substance	:	MRD-79-10 (raw naphthenic acid derived from kerosene)
Method	:	
Method detail	:	Rats were observed at 1, 2, 4 and 6 hours after dosing and then daily for 14 days. Mortality, toxicity, and pharmacological effects were recorded. Body weights were recorded at pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross

pathology.

Result	:	Deaths occurred at dose levels of 3.16 g/kg and higher. Significant pre-death toxic signs included tremors, lethargy, ptosis, ataxia, prostration, negative righting reflex, flaccid muscle tone, piloerection, diarrhea, chromodacryorrhea, dyspnea and chromorhinorrhea. Body weight changes were noted in the survivors. Significant necropsy findings in the animals that died included dilated hearts and gastrointestinal irregularities.
Remark	:	Other data for rats includes an LD50 of 3.0 g/kg bw for naphthenic acid fraction from crude kerosene acids and 5.2 g/kg bw for naphthenic acid fraction from mixed crude oils (Rockhold, 1955, as cited in Appendix C). An oral acute toxicity test in rats with a mixture of naphthenic acids isolated from Athabasca oil sands produced appetite suppression, hepatoxicity and cardiovascular effects with a single dose of 300 mg/kg. (Rogers, V.V., et al., Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355). An LD50 of 3.55 g/kg for mice was reported by Pennisi and Lynch, 1977 (as cited in Appendix C).
Reliability	:	[1] Reliable without restrictions, as assessed in Appendix C.
Reference	:	Exxon, 1979. Acute Oral Toxicity of MRD-79-10 in Rats, MB 79-3702, as cited in Appendix C.

5.1.2 ACUTE INHALATION TOXICITY

Type	:
Guideline/method	:
Species	:
Strain	:
Sex	:
Number of animals	:
Vehicle	:
Concentrations	:
Exposure time	:
LC50	:
Year	:
GLP	:
Test substance	:
Method	:
Method detail	:
Result	:
Remark	:
Reliability	:
Reference	:

5.1.3 ACUTE DERMAL TOXICITY

Type	:	Acute dermal LD50 with irritation
Guideline/method	:	
Species	:	Rabbit
Strain	:	New Zealand White
Sex	:	Male and female
Number of animals	:	2 per sex
Vehicle	:	None – administered undiluted
Doses	:	3.16 g/kg
LD50	:	> 3.16 g/kg
Year	:	1979
GLP	:	Unknown
Test substance	:	MRD-79-10 (raw naphthenic acid derived from kerosene)

Method	:	
Method detail	:	The test substance was applied dermally to the clipped abraded abdomens of each animal. The area was covered with gauze and secured by a thick plastic binder, which was removed after 24 hours, and the skin washed with water or corn oil. Animals were then observed for mortality and toxic effects at 2 and 4 hours, and once daily thereafter. Body weight was recorded before and after the test. Dermal irritation was recorded at 1, 3, 7, 10 and 14 days. Mortality, toxicity and pharmacological effects were observed at 1, 2, 4, and 6 hours after dosing and once daily for 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.
Result	:	No deaths occurred. Symptoms of toxicity appeared 2 to 4 hours after dosing and 3 out of 4 animals showed signs of toxicity until day 12 or 13. During the first five days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The test substance was judged to be moderately to severely irritating to the occluded skin. Mean values for erythema and edema at intact sites were 1.69 and 1.3, respectively.
Remark	:	
Reliability	:	[1] Reliable without restriction, as assessed in Appendix C.
Reference	:	Exxon, 1979. Acute Dermal Toxicity of MRD-79-10 in Rabbits, MB 79-3702, as cited in Appendix C.

5.2.1 SKIN IRRITATION

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
Classification	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	Moderately to severely irritating to rabbits.
Remark	:	See results of acute dermal LD50 study, described above.
Reliability	:	
Reference	:	

5.2.2 EYE IRRITATION

Type	:	Eye irritation
Guideline/method	:	
Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male and female
Concentration	:	
Dose	:	
Exposure time	:	
Number of animals	:	3 per sex

Vehicle	:	None – administered undiluted
Classification	:	
Year	:	1979
GLP	:	Unknown
Test substance	:	MRD-79-10 (raw naphthenic acid derived from kerosene)
Method	:	
Method detail	:	0.1 mL of test substance was placed into the conjunctival sac of the eye of each of the six rabbits. The untreated eye served as a control. Animals were observed at 1 and 4 hours, and on days 1, 2, 3, 4 and 7. If a positive score was noted on day 7, ocular readings were scored on day 10. If an positive score was noted on day 10, observations were made on day 14. Fluorescein was used in examining ocular reactions on day 3 and after. The Draize technique was used as the scoring system.
Result	:	One animal had a positive corneal score on days 1 and 2; one animal had a positive iris score at hours 1 and 4. All animals exhibited positive conjunctival scores at some point during the first three days of observation. By day 4, no animals showed positive scores. The test material was judged to be an irritant. In a later summary report, eye irritation was judged to be moderate.
Remark	:	
Reliability	:	[1] Reliable without restrictions, as assessed in Appendix C.
Reference	:	Exxon, 1979. Eye Irritation Study of MRD-79-10 in Rats, MB 79-3702, as cited in Appendix C.

5.4 REPEATED DOSE TOXICITY

Type	:	Oral 90-d subchronic toxicity test
Guideline/method	:	
Species	:	Rat
Strain	:	Wistar
Sex	:	Female
Number of animals	:	12 per dose level
Route of admin.	:	Oral gavage
Exposure period	:	
Frequency of treatment	:	1 dose/day, 5 days/week
Post exposure period	:	
Dose	:	0.6, 6, or 60 mg/kg bw (aqueous solutions of naphthenic acids)
Control group	:	Yes (7 ml tap water)
NOAEL	:	6 mg/kg/day
LOAEL	:	60 mg/kg/day (5 doses per week)
Other	:	
Year	:	2002
GLP	:	Unknown
Test substance	:	Mixture of naphthenic acids (acyclic and 1-, 2-, 3-, and 4-ringed compounds, administered as sodium salt solutions) isolated from tailings pond water from Athabasca oil sands
Method	:	
Method detail	:	Animals were monitored daily. Changes in body weight, food consumption and behavioral or clinical signs recorded. Blood samples were collected from the ventral tail vein on day 45 of dosing and analyzed for plasma biochemical and hematological effects. Blood samples were similarly analyzed from cardiac punctures on day 91. Following euthanization, the liver, kidney, spleen, heart, lung and ovaries were examined.
Result	:	Significant physical, clinical, and pathological changes at a dose level of 60 mg/kg/day (5 doses per week). No significant adverse effects were seen at a dose level of 6 mg/kg/day. Several parameters suggested that the liver

		was the primary target organ in this study. Liver weight was increased 35% above control values in the high dose group. Body weight gain was also reduced 8-9% in this exposure group compared to controls. Plasma cholesterol was reduced and amaylase activity increased in the high dose group.
Remark	:	
Reliability	:	[2] Reliable with restriction. Only female rats were used and a limited number of organs examined.
Reference	:	Rogers et al. 2002. Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355.

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Mutagenicity
Guideline/method	:	Ames assay
System of testing	:	Bacteria *in vitro*
Species	:	*Salmonella typhimurium*
Strain	:	TA100, TA1535, TA97, TA98
Test concentrations	:	1 – 1000 ug/L depending upon strain
Cytotoxic concentr.	:	
Metabolic activation	:	With and without
Year	:	1993
GLP	:	Yes
Test substance	:	Calcium naphthenate
Method	:	
Method detail	:	Activation was with induced male Sprague Dawley rat liver S9 and induced male Syrian hamster liver S9.
Result	:	Negative
Remark	:	Sodium naphthenate and copper naphthenate were also negative in the *Salmonella* mutagenicity test, performed similarly (National Toxicology Program, http://ntp-server.niehs.nih.gov).
Reliability	:	[1] Reliable without restriction
Reference	:	Study ID A21560, National Toxicology Program (http://ntp-server.niehs.nih.gov)

Type	:	In vitro cytogenetics
Guideline/method	:	
System of testing	:	
Species	:	
Strain	:	
Test concentrations	:	
Cytotoxic concentr.	:	
Metabolic activation	:	
Year	:	
GLP	:	Yes
Test substance	:	Sodium naphthenate
Method	:	
Method detail	:	
Result	:	Negative results were obtained for chromosome aberrations, while positive results were obtained for Sister Chromatid Exchanges.
Remark	:	
Reliability	:	[1] Reliable without restriction
Reference	:	Study ID 058122, National Toxicology Program (http://ntp-server.niehs.nih.gov)

Type	:	Mutagenicity

Guideline/method	:	
System of testing	:	
Species	:	Mouse Lymphoma
Strain	:	L5178Y (TK+/TK-)
Test concentrations	:	0.005 – 0.037 UL/ML
Cytotoxic concentr.	:	
Metabolic activation	:	none
Year	:	
GLP	:	
Test substance	:	Naphthenic acid, calcium salt (61789-36-4)
Method	:	Suspension Plate
Method detail	:	
Result	:	Positive
Remark	:	
Reliability	:	[2] Reliable with restrictions.
Reference	:	Short-term test program sponsored by the Division of Cancer Biology, National Cancer Institute, Dr. Shen Yang, Project Officer. Cited in Chemical Carcinogenesis Research Information System, National Library of Medicine:Record #1169 (http://toxnet.nlm.nih.gov)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Route of admin.	:	
Exposure period	:	
Doses	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

5.8.2 DEVELOPMENTAL TOXICITY

Type	:	
Guideline/method	:	
Species	:	Rat
Strain	:	Wistar
Sex	:	Female
Route of admin.	:	Oral
Exposure period	:	Pre-breeding, breeding and gestation (no other details provided)
Frequency of treatment	:	daily
Duration of test	:	
Doses	:	0.6, 6, and 60 mg/kg bw
Control group	:	
NOAEL maternal tox.	:	
NOAEL teratogen.	:	
Other	:	

Other	:	
Other	:	
Year	:	2002
GLP	:	unknown
Test substance	:	Naphthenic acids isolated from Athabasca oil sands tailings
Method	:	
Method detail	:	
Result	:	Fetal malformation were not observed in any offspring.
Remark	:	
Reliability	:	[4] Not assignable, as assessed in Appendix C.
Reference	:	Rogers, V.V., et al., 200b, as cited in Appendix C.

5.8.3 TOXICITY TO REPRODUCTION

Type	:	Dermal exposure
Guideline/method	:	
In vitro/in vivo	:	In vivo
Species	:	12 male New Zealand White rabbits
Strain	:	
Sex	:	Male
Route of admin.	:	Dermal
Exposure period	:	6 hours/day
Frequency of treatment	:	5 days/week
Duration of test	:	
Doses	:	2 ml undiluted material
Control group	:	12 male
Year	:	1984
GLP	:	
Test substance	:	An over-based calcium naphthenate in mineral oil (SAP-011)
Method	:	
Method detail	:	
Results	:	A group of 12 male New Zealand White rabbits was dermally exposed to 2 ml of undiluted SAP 011 for 6 hours daily for 5 days each week over a 10-week period. Following the exposure period, each male rabbit was mated with two untreated female rabbits. Males were subsequently necropsied and their reproductive tracts examined macroscopically and microscopically. Female rabbits were necropsied on day 29 of gestation and examined for reproductive parameters. Study results showed no adverse effects on reproductive performance due to male exposure. There were no adverse signs of toxicity either systemically or at the site of application in treated males, as well as no pathological findings of the reproductive tract that could be related to SAP 011 exposure.
Remark	:	
Reliability	:	[2] Reliable with restrictions
Reference	:	Dix, K.M. and S.L. Cassidy. 1983. Toxicity studies on oil additives: one generation reproduction study in male rabbits repeatedly treated dermally with SAP 0111 for 10 weeks. External Report SBER.84.002. Shell Research Ltd. (NTIS No. OTS0507494)

Type	:	One generation reproduction
Guideline/method	:	
Species	:	Rat
Strain	:	Wistar
Sex	:	Female
Route of admin.	:	Oral
Exposure period	:	Pre-breeding, breeding, and gestation – no other details provided

Frequency of treatment	:	Daily
Duration of test	:	
Doses	:	0.6, 6, and 60 mg/kg bw
Control group	:	yes
Year	:	2002
GLP	:	unknown
Test substance	:	Naphthenic acid isolated from Athabasca oil sands tailings
Method	:	
Method detail	:	
Results	:	Dramatic effects were seen on female fertility at 60 mg/kg/day. While the control and 6 mg/kg/day dose groups achieved 93 and 100% reproductive success, respectively, only 7% of the females in the high dose group successfully bore a litter. Total cholesterol in this group was 30% lower than in the controls. Mating and ovulation were unaffected. Results suggest that dose-related infertility may be associated with poor embryonic implantation, an effect that might be secondary to depressed sex hormone production requiring cholesterol as a precursor.
Remark	:	
Reliability	:	[2] Not assignable, as assessed in Appendix C.
Reference	:	Rogers, V.V. et al, 2002. As cited in Appendix C.

6.0 OTHER INFORMATION

6.1 Carcinogenicity

In a study in which calcium naphthenate was dermally administered to female mice (two times per day for two years), twelve epidermal and one dermal tumor at the treated sites were observed in eight of the exposed mice. Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present. (Appendix C).

6.2 Skin sensitization

1. General Information

ID 7646-79-9

Date January 31, 2005

201-16127B

1.0 SUBSTANCE INFORMATION

Generic Name	:	Cobalt chloride
Chemical Name	:	Cobaltous chloride
CAS Registry No.	:	7646-79-9
Component CAS Nos.	:	
EINECS No.	:	
Structural Formula	:	$CoCl_2$
Molecular Weight	:	129.84
Synonyms and Tradenames	:	Cobalt(II) chloride; Cobalt dichloride
References	:	ATSDR, 2001. Draft Toxicological Profile for Cobalt, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR), September 2001. (This reference is subsequently listed in this document as ATSDR Sept 2001 Draft).

2.1 MELTING POINT

Type	:	
Guideline/method	:	
Value	:	735 °C
Decomposition	:	at °C
Sublimation	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Decomposes at 400 °C on long heating in air
Reliability	:	2 (reliable with restrictions): Source is well established data compendium.
Reference	:	O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ

2.2 BOILING POINT

Type	:	
Guideline/method	:	
Value	:	1,049 °C
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	2 (reliable with restrictions): Source is well established data compendium.
Reference	:	O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ

2.3 DENSITY

Type	:	
Guideline/method	:	
Value	:	3.367 at 25 °C
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	2 (reliable with restrictions): Source is well established data compendium.
Reference	:	O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ

2.4 VAPOR PRESSURE

Type	:	
Guideline/method	:	
Value	:	hPa at °C
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

2.5 PARTITION COEFFICIENT

Type	:	
Guideline/method	:	
Partition coefficient	:	
Log Pow	:	at °C
pH value	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Not applicable – metal dissociates (ionizes) in water
Reliability	:	
Reference	:	

2.6.1 SOLUBILITY IN WATER

Type	:	
Guideline/method	:	
Value	:	450 g/L at 7 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at °C
Description	:	
Stable	:	
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	
Deg. products CAS#	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	544 g/L in ethanol; 86 g/L in acetone
Reliability	:	2 (reliable with restrictions): Source is well established data compendium
Reference	:	Weast. R.C. (ed.). 1988-1989. Handbook of Chemistry and Physics. 69th Ed. CRC Press Inc., Boca Raton, FL., p. B-86.

2.7 FLASH POINT

Type	:	
Guideline/method	:	
Value	:	°C
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

3.1.1 PHOTODEGRADATION

Type				
Guideline/method	:			
Light source	:			
Light spectrum	:			
Relative intensity	:	based on		
Spectrum of substance	:	lambda (max, >295nm)	:	
		epsilon (max)	:	
		epsilon (295)	:	
Conc. of substance	:		at	°C
DIRECT PHOTOLYSIS				
Halflife (t1/2)	:			
Degradation	:	% after		
Quantum yield	:			
INDIRECT PHOTOLYSIS				
Sensitizer	:			
Conc. of sensitizer	:			
Rate constant	:			
Degradation	:			
Deg. product	:			
Year	:			
GLP	:			
Test substance	:			
Deg. products CAS#	:			
Method	:			
Method detail	:			
Result	:			
Remark	:	Not applicable – metal does not degrade		
Reliability	:			
Reference	:			

3.2.1 MONITORING DATA

Type of measurement	:
Media	:
Concentration	:
Substance measured	:
Method	:
Method detail	:
Result	:
Remark	:
Reliability	:
Reference	:

3.3.1 TRANSPORT (FUGACITY)

Type	:	
Media	:	
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Year	:	
Test substance	:	
Method	:	

Method detail :
Result :
Remark :
Reliability :
Reference :

3.5 BIODEGRADATION

Type :
Guideline/method :
Inoculum :
Concentration : related to
related to
Contact time :
Degradation : (±) % after day(s)
Result :
Kinetic of test subst. : % (specify time and % degradation)
%
%
%
%
Control substance :
Kinetic : %
%
Deg. product :
Year :
GLP :
Test substance :
Deg. products CAS# :
Method :
Method detail :
Result :
Remark : Not applicable – the metal will not degrade
Reliability :
Reference :

3.7 BIOCONCENTRATION

Type :
Guideline/method :
Species :
Exposure period : at °C
Concentration :
BCF :
Elimination :
Year :
GLP :
Test substance :
Method :
Method detail :
Result :
Remark :
Reliability :
Reference :

4.1 ACUTE TOXICITY TO FISH

Type	:	Acute
Guideline/method	:	Flow-through, freshwater
Species	:	Rainbow trout (*Onchorhynchus mykiss*)
Exposure period	:	96 hr
NOEC	:	
LC0	:	
LC50	:	1.41 mg Co/L (95% C.I. = 0.57 – 3.47 mg Co/L)
LC100	:	
Other	:	LC20 = 0.53 mg Co/L (95% C.I. = 0.24 – 1.20 mg Co/L)
Other	:	Incipient lethal level for 50% mortality (time independent) = 0.35 mg Co/L
Other	:	144-hr LC50 = 0.52 mg Co/L (95% C.I. = 0.29 – 0.95 mg Co/L)
Limit test	:	
Analytical monitoring	:	Yes (results based on measured concentrations)
Year	:	1998
GLP	:	No
Test substance	:	Cobalt chloride dihydrate ($CoCl_2 \cdot 2H_20$)
Method	:	
Method detail	:	Tests were conducted with trout fry in water with an alkalinity and hardness of approximately 25 mg $CaCO_3$/L. Exposure concentrations ranged from 0.125 to 2.0 mg Co/L. Exposures were continued for up to 14 days, with mortality assessed every 2 hr for the first 48 hr, and every 6 h thereafter.
Result	:	The onset of mortality was slow (48 hr or greater), generally not reaching a plateau for 200 hr or more.
Remark	:	Study data indicate that the rainbow trout is highly sensitive to the toxic effects of cobalt. For comparison, reported 96-h LC50 values for other fish species include 22.0 mg Co/L for the fathead mninnow (*Pimephales promelas*), 333 mg Co/L for the carp (*Cyprinus carpio*), and 275 mg Co/L for the mummichog (*Fundulus heteroclitus*) (U.S. EPA, ECOTOX data base, 2003). Available data suggest that toxicity to fish is reduced with increasing hardness up to a hardness of approximately 400 mg $CaCO_3$/L (Diamond, J. et al., 1992. Aquat. Toxicol., 22:163-180).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study
Reference	:	Marr, J.C.A., J.A. Hansen, J.S. Meyer, D. Cacela, T. Podrabsky, J. Lipton, and H.L. Bergman. 1998. Toxicity of cobalt and copper to rainbow trout: application of a mechanistic model for predicting survival. Aquat. Toxicol., 43(4):225-238.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	Acute
Guideline/method	:	Static, freshwater
Species	:	*Daphnia magna* (water flea)
Exposure period	:	48 hr
NOEC	:	
EC0	:	
EC50	:	1.52 mg Co/L (95% C.I. = 1.01 - 2.28 mg Co/L)
EC100	:	
Other	:	24 hr LC50 = 2.11 mg Co/L (95% C.I. = 1.49 - 3.05 mg Co/L)
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	No
Year	:	1987
GLP	:	No

Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	American Public Health Association (APHA), 1976, Standard Methods for the Examination of Water and Wastewater.
Method detail	:	Tests were conducted in well water with a total hardness of 240 mg $CaCO_3$/L and a total alkalinity of 400 mg $CaCO_3$/L. Solutions were not renewed during the test. Daphnids were not fed during the test.
Result	:	
Remark	:	In an older study, the 48-hr LC50 for *Daphnia magna* has been reported as 5.5 mg Co/L (Cabejszek and Stasiak, 1960 as cited in the U.S. EPA ECOTOX database, 2003). The 48-hr LC50 for another daphnid, *Daphnia hyaline*, has been reported as 1.52 mg Co/L (Baudouin and Scoppa, 1974 as cited in the U.S. EPA ECOTOX database, 2003). Others have found 48-hr LC50 values for *Ceriodaphnia dubia* of 2.35, 4.60, and 4.20 mg Co/L for tests conducted with water hardness of 50, 200, and 400 mg $CaCO_3$/L, respectively (Diamond, J. et al., 1992. Aquat. Toxicol., 22:163-180).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study
Reference	:	Khangarot, B.S., P.K. Ray, and H. Chandra. 1987. *Daphnia magna* as a model to assess heavy metal toxicity: comparative assessment with mouse system. Acta. Hydrochim. Hydrobiol., 15(4): 427-432.

4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

Type	:	Algal growth assay
Guideline/method	:	Static, freshwater
Species	:	*Chlorella vulgaris* (green algae)
Endpoint	:	Population growth
Exposure period	:	96 hr
NOEC	:	
LOEC	:	
EC0	:	
EC10	:	
EC50	:	0.52 mg Co/L (95% C.I. = 0.48 – 0.56 mg Co/L)
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	No
Year	:	1993
GLP	:	
Test substance	:	Cobalt chloride
Method	:	
Method detail	:	Tests conducted in modified Bristol's medium (pH 6.5) with a 16:8 day/night photoperiod (280 foot candles). Cultures were incubated at 19°C ± 1°C. Results were based on experiments run in triplicate.
Result	:	Growth was 63.8% and 28.4% of controls at concentrations of 0.32 and 1.00 mg Co/L, respectively.
Remark	:	Other aquatic plants are much less sensitive to cobalt. The reported 96-h EC50 for *Spirulina platensis* (blue-green algae) is 23.8 mg Co/L (Sharma et al., 1987 as cited in the U.S. EPA ECOTOX database, 2003). The 7-d IC50 for *Lemna minor* (duckweed) is 16.9 mg Co/L (Dirilgen and Inel, 1994 as cited in the U.S. EPA ECOTOX database, 2003).
Reliability	:	2 (reliable with restrictions); comparable to guideline study
Reference	:	Rachlin, J.W. and A. Grosso. 1993. The growth response of the green alga *Chlorella vulgaris* to combined divalent cation exposure. Arch. Environ. Contam. Toxicol., 24: 16-20.

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In vitro/in vivo	:	
Type	:	
Guideline/method	:	
Species	:	
Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Route of administration	:	
Exposure time	:	
Product type guidance	:	
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	1st: 2nd: 3rd:
Toxic behavior	:	
Deg. product	:	
Deg. products CAS#	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Absorption of cobalt in the digestive tract is influenced by the chemical form of the metal, with increasing solubility resulting in increasing absorption (ATSDR Sept 2001 Draft). Approximately 13-34% of cobalt chloride, a soluble form, is absorbed in the gut of rats, but absorption in the gut may be increased in iron deficient individuals. Following oral exposure, cobalt is eliminated primarily in feces and secondarily in urine. For the more soluble forms of cobalt, e.g., cobalt chloride, 70 – 80% of the administered dose is eliminated in the feces. For absorbed cobalt, elimination is rapid primarily in the urine (Barceloux, D.G. 1999. Cobalt. Clin. Tox. 37:201-206). Elimination is biphasic or triphasic. The terminal phase involves a very small residual level of cobalt and has a half-life in years (ATSDR Sept 2001 Draft).
Reliability	:	
Reference	:	

5.1.1 ACUTE ORAL TOXICITY

Type	:	Oral
Guideline/Method	:	Not specified
Species	:	Rat
Strain	:	Wistar
Sex	:	Male and female
Number of animals	:	5 per sex per dose level
Vehicle	:	Distilled water
Doses	:	50, 600, 720, 864, and 1137 mg/kg

LD50	:	766 mg/kg as compound (hexahydrate); 95% C.I. = 677 – 867 mg/kg) 190 mg/kg as cobalt
Year	:	1982
GLP	:	No
Test substance	:	Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	Single dose administered by gastric incubation
Method detail	:	Mortality assessed after a 10-d observation period.
Result	:	
Remark	:	Reported LD50s of cobalt chloride to rats range from 42.4 to 190 mg $CoCl_2$/kg bw (equivalent to 19.8 to 85.5 mg Co/mg bw) (ATSDR Sept 2001 Draft). Toxicity of cobalt sulfate is reported to be similar to that of the chloride with oral LD50s for rats ranging from 123 to 161 mg/kg bw (equivalent to 46.7 to 61.2 mg Co/kg b.w.) (ATSDR Sept 2001 Draft). For the mouse, LD50 values are 89.3 and 123 mg/kg for cobalt chloride and cobalt sulfate, respectively, which are equivalent to 40.2 and 46.7 mg Co/kg b.w. when expressed as the metal only (ATSDR Sept 2001 Draft).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Speijers, G.J.A., E.I. Krajnc, J.M. Berkvens, and M.J. van Logten. 1982. Acute oral toxicity of inorganic cobalt compounds in rats. Food Chem. Toxicol., 20:311-314.

5.1.2 ACUTE INHALATION TOXICITY

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	
LC50	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	No acute toxicity studies have been located for this compound.
Reliability	:	
Reference	:	

5.1.3 ACUTE DERMAL TOXICITY

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
LD50	:	
Year	:	

GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Increased proliferation of lymphatic cells was seen in rats, mice and guinea pigs dermally exposed to cobalt chloride in DMSO once per day for 3 consecutive days, with LOAEL values ranging from 9.6 to 14.7 mg Co/kg/day (Ikarashi, Y., et al., 1992. Toxicology, 76:283-292). Stimulation indices of 3 or greater (indicative of a significant response by the authors), were reported for mice exposed to 1, 2.5 or 5% $CoCl_2$ (equivalent to 10.8, 27, or 54.1 mg Co/kg/day), rats exposed to 2.5 or 5% $CoCl_2$ (equivalent to 9.6 or 19.2 mg Co/kg/day), and guinea pigs exposed to 5% $CoCl_2$ (equivalent to 14.7 mg Co/kg/day).
Reliability	:	
Reference	:	

5.2.1 SKIN IRRITATION

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
Classification	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Dermatitis is a common result of dermal exposure to cobalt in humans and has been verified in a large number of studies (ATSDR Sept 2001 Draft). The dermatitis is probably caused by an allergic reaction to cobalt.
Reliability	:	
Reference	:	

5.2.2 EYE IRRITATION

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Concentration	:	
Dose	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
Classification	:	
Year	:	
GLP	:	

Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

5.4 REPEATED DOSE TOXICITY

Type	:	Repeated dose
Guideline/method	:	Oral
Species	:	Rat
Strain	:	Not specified
Sex	:	Male
Number of animals	:	30
Route of admin.	:	Oral via stomach tube
Exposure period	:	150 to 210 days
Frequency of treatment	:	Five days per week
Post exposure period	:	0 to 30 days
Doses	:	4 or 10 mg Co/kg
Control group	:	Yes
NOAEL	:	
LOAEL	:	4 mg Co/kg (organ weights increased)
Other	:	
Year	:	1959
GLP	:	No
Test substance	:	Cobalt chloride
Method	:	
Method detail	:	The erythrocyte count, hemoglobin and hematocrit determinations were performed at frequent intervals for animals receiving 10 mg Co/kg. At study termination, all rats were sacrificed, organs examined and weighed, and sections made histological examination.
Result	:	The average weights of kidneys, livers, and spleens of the cobalt-treated groups were slightly heavier than the controls. Cobalt exposure at 10 mg/kg produced significant polycythemia. Histological examination of the kidneys revealed necrosis of the linings of the tubules in rats treated with 10 mg Co/kg, but not in those of the 4 mg Co/kg group. The effects was reversible, however, as examination of kidneys of rats autopsied 30 days after cobalt administration was discontinued showed no necrosis and were normal compared to the kidneys from control rats.
Remark	:	Results are highly consistent with those reported by others. Repeated oral dosing of rats with cobalt chloride at levels ranging from 0.5 to 30.2 mg Co/kg/day (as cobalt chloride) for periods ranging from 12-16 days up to 7 months resulted in the following observations associated with LOAELs: reduced weight gain, increases in some organ weights (heart, liver and lungs); increased hematocrit, hemoglobin, and red blood cells; renal tubular necrosis; and various changes on cardiac physiology (left ventricular hypertrophy, impaired ventricular function, and degeneration of myofibrils) (ATSDR Sept 2001 Draft). Cardiac effects were observed in rats at LOAEL's ranging from 8.4 to 12.4 mg Co/kg/day, for cobalt sulfate or cobalt chloride, with exposure periods of 3 weeks to 6 months (ATSDR Sept 2001 Draft).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Murdock, H.R. 1959. Studies on the pharmacology of cobalt chloride. J. Amer. Pharm. Assoc., 48:140-142.

Type	:	Repeated dose
Guideline/method	:	Not specified
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male
Number of animals	:	4
Route of admin.	:	Oral
Exposure period	:	8 weeks
Frequency of treatment	:	Daily
Post exposure period	:	None
Doses	:	2.5, 10, or 40 mg/kg (equivalent to 0.6, 2.5, or 10 mg Co/kg)
Control group	:	Yes
NOAEL	:	0.6 mg Co/kg
LOAEL	:	2.5 mg Co/kg (hemoglobin, red blood cell count)
Other	:	
Year	:	1947
GLP	:	No
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	
Method detail	:	Cobalt was administered orally in a gelatin capsule (mixed in equal part of wheat flour and powdered sugar). Blood counts and hemoglobin determinations were made at the start of the test and at two week intervals.
Result	:	Hemoglobin content and numbers of erythrocytes were increased in rats receiving either 2.5 or 10 mg Co/kg/day, but not in those receiving 0.6 mg Co/kg/day.
Remarks	:	Other researchers have reported similar results in long-term studies with rats although many study details are lacking in the published report (Krasovskii, G.N. and S.A. Fridlyand. 1971. Hyg. Sanit., 26:277-279). They found that oral doses of 0.5 and 2.5 mg Co/kg six days per week for seven months stimulated hemopoiesis and decreased immunological reactivity (reduced the phagocytic index). Daily doses of 0.5 mg Co/kg and greater also produced mild to moderate increases in conditioned flexes. However, daily doses of 0.05 mg Co/kg had no effects on the indices investigated. Others have also reported the neurotoxic and behavior effects of cobalt on rats after chronic dietary exposures (Nation, J.R. et al., 1983. Neurobehav. Toxicol. Teratol., 5:9-15).
Reliability	:	2 (reliable with restrictions): Documentation was incomplete; however, the results are highly consistent with others in the scientific literature.
Reference	:	Stanley, A.J., H.C. Hopps, and A.M. Shideler. 1947. Cobalt polycythemia. II. Relative effects of oral and subcutaneous administration of cobaltous chloride. Proc. Soc. Exp. Biol. Med., 66:19-20.

5.5 GENETIC TOXICITY - MUTAGENICITY

Type	:	Mutagenicity
Guideline/method	:	Ames Assay
System of testing	:	Bacteria *in vitro*
Species	:	*Salmonella typhimurium* LT2
Strains	:	TA100
Test concentrations	:	10^{-4} to 10^{-1} M
Cytotoxic concentr.	:	10^{-2} M
Metabolic activation	:	No
Year	:	1981
GLP	:	No
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)

Method	:	Ames, B.N., J. McCann, and E. Yamasaki. 1975. Mutat. Res., 31:347-364.
Method detail	:	
Result	:	Negative both above and below the cytotoxic concentration
Remark	:	Cobalt compounds with a valence state of II, the form of cobalt released by dissociation of cobalt chloride, are generally nonmutagenic in *in vitro* bacterial assays (ATSDR Sept 2001 Draft). For example, cobalt chloride was not mutagenic in plate incorporation and fluctuation assays with *Salmonella* TA strains or a *Escherichia coli* WP2 strain (Arlauskas, A., et al., 1985. Environ. Res., 36:379-388). However, a weak positive mutagenic response has been found in the rec assay with *Bacillus subtilis* at a concentration of 0.05 M (Kanematsu, N. et al., 1980. Mutat. Res., 77:109-116). A very weak positive response has also been found in Chinese hamster V79 cells, but only at a highly cytotoxic concentration (Miyaki, M. et al. 1979. Mutat. Res., 68: 259-263).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Tso, W-W. and W-P Fung. 1981. Mutagenicity of metallic cations. Toxicolog. Lett., 8:195-200.

Type	:	Mutagenicity
Guideline/method	:	Ames Assay
System of testing	:	Bacteria *in vitro*
Species	:	*Salmonella typhimurium* LT2
Strains	:	TA98, TA100, TA1537, and TA2637
Test concentrations	:	0.1 to 1,000 μM/plate
Cytotoxic conc.	:	Not specified
Metabolic activation	:	No
Year	:	1986
GLP	:	No
Test substance	:	Cobalt chloride
Method	:	Ames, B.N., J. McCann, and E. Yamasaki. 1975. Mutat. Res., 31:347-364.
Method detail	:	A modified Tris-HCl minimal medium with low phosphate content was used to prevent formation of insoluble metal phosphates in the test system.
Result	:	Negative
Remark	:	Although cobalt chloride alone did not produce mutants in this test system, it was mutagenic when it was added as a mixture with one of several heteroaromatic compounds (e.g., 4-aminoquinoline, 9-aminoacridine). The enhanced mutagenicity was attributed by the authors to the formation of weak to moderate complexes between these chemicals and the Co(II) cation, which may have enhanced transmembrane permeation or intercellular binding.
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Ogawa, H.I., K. Sakata, T. Inouye, S. Jyosui, Y. Niyitani, K. Kamimoto, M. Morishita, S. Tsuruta, and Y. Kato. 1986. Combined mutagenicity of cobalt(II) salt and heteroaromatic compounds in *Salmonella typhimurium*. Mutat. Res., 172: 97-104.

5.6 GENETIC TOXICITY - CLASTOGENICITY

Type	:	Chromosomal aberrations in bone marrow cells
Guideline/method	:	*In vivo*
Species	:	Mouse (*Mus musculus*)
Strain	:	Swiss albino
Sex	:	Male
Route of admin.	:	Oral (single dose)
Exposure period	:	6, 12, 18, or 24 hr.
Dose	:	20, 40 , or 80 mg/kg b.w.
Year	:	1991
GLP	:	No
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	Preston, R.J. et al., 1987. Mutat. Res., 189:157.
Method detail	:	Test compound was administered orally to five animals per dose group. Mice were 6-8 weeks old at that time. Colchicine (0.04%) was injected i.p. at 90 min prior to sacrifice. Bone marrow cells were removed form femurs by flushing with 0.8% sodium citrate. From each animal, 50 well-scattered metaphase plate were scored for chromosomal aberrations. Abnormalities were scored separately as total aberrations (with and without gaps) and as breaks per cell.
Result	:	Administration of cobalt chloride produced a concentration-dependent increase in total chromosomal aberrations.
Remark	:	Cobalt compounds, including soluble salts, are observed to be clastogenic (cause chromosomal aberrations) in a range of mammalian assay systems. For example, increased micronucleus formation was observed following i.p. injection of 12.4 and 22.3 mg Co/kg (as cobalt chloride), but not after injection of 6.19 mg Co/kg (NOEL) (ATSDR Sept 2001 Draft). There is evidence that soluble cobalt(II) cations exert a genotoxic activity in vitro and in vivo in experimental systems, but evidence in humans is lacking (Lison, D. et al., 2001. Occup. Environ. Med., 58: 619-625).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Palit, S., A. Sharma, and G. Talukder. 1991. Chromosomal aberrations induced by cobaltous chloride in mice in vivo. Biol. Trace Elem. Res., 29:139-145.

Type	:	Micronucleus Test
Guideline/method	:	In vivo
Species	:	Mouse
Strain	:	BALB/c AnNCRj
Sex	:	Male
Route of admin.	:	Intraperitoneally
Exposure period	:	30 hr
Doses	:	25, 50, or 90 mg Co/kg b.w.
Year	:	1993
GLP	:	No
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	Von Ledbur, M. and W. Schmid. 1973. Mutat. Res., 19:109-117.
Method detail	:	Mice were injected once ip and sacrificed after 30 hr. Bone marrow smears were prepared and stained. The incidence of micronucleated polychromatic erythrocytes (MPCE) was determined in 1,000 cells. In addition, the ratio of polychromatic erythrocytes (P) to normochromatic erythrocytes (N) was determined in 2,000 erythrocytes.
Result	:	Treatment with cobalt induced a dose-dependent increase in the frequency of MPCE. The P/N ratio was significantly reduced ($P<0.05$) in mice dosed at 90 mg/kg b.w.

Remark	:	This study also included an *in vitro* micronucleus test with mouse bone marrow cells, both with and without metabolic activation with an S9 fraction. In contrast to the *in vivo* test, the *in vitro* test did not produce any significant changes in frequency of MPCE or the P/N ratio at dose levels of cobalt chloride hexahydrate up to 50 mg/L in the cell suspension.
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Suzuki, Y., H. Shimizu, Y. Nagae, M. Fukumoto, H. Okonogi, and M. Kadokura. 1993. Micronucleus test and erythropoiesis: effect of cobalt on the induction of micronuclei by mutagens. Environ. Mol. Mutagen., 22:101-106.
Type	:	DNA damage in isolated human lymphocytes
Guideline/method	:	Alkaline Comet Assay (*in vitro*)
Species	:	Human
Strain	:	
Sex	:	Female
Route of admin.	:	In vitro
Exposure period	:	15 min
Doses	:	0.3, 0.6, 1.2, 1.5, 2.0, 2.5, 3.0, and 6.0 mg Co/L
Year	:	1998
GLP	:	No
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	The alkaline comet assay performed using a modification of the method of Singh et al. 1988. Exp. Cell. Res., 175:184-191.
Method detail	:	Tests were conducted on lymphocytes taken from two healthy female donors. Cells were for 15 min exposed after 24 of stimulation by phytohaemagglutinin. After treatment, the cells were centrifuged for 10 min at 400 g. The supernatant was removed and the cell pellet was resuspended and processed for the alkaline comet assay (single cell electrophoresis assay). Fifty or 100 randomly selected slides were analyzed, with tail length, tail DNA, and tail movement recorded.
Result	:	There was considerable interexperimental and interdonor variability in data; however, at the highest dose level (6.0 mg Co/L) there was a statistically significant increase in tail movement in all experiments, indicating DNA damage (single strand breaks and alkali labile sites). Tail movement was also increased at lower doses, but did not show a clear dose-dependent trend.
Remark	:	Using human lymphocytes and macrophages ($P388D_1$ cells), an increase in sister chromatid exchanges (SCE) after exposure to cobalt chloride at 10^{-4} to 10^{-5} M has been also demonstrated (Andersen, O. 1983. Environ. Health Perspect., 47: 239-253). Others have also found that cobalt chloride increases DNA strand breaks in human diploid fibroblasts and Chinese hamster ovary cells after *in vitro* exposures, although only when determined by alkaline sediment sucrose velocity sedimentation and not when measured by nucleoid sedimentation or nick translation assays (Hamilton-Koch, W. et al., 1986. Chem.-Biol. Interactions, 59:17-28).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	De Beck, M., D. Lison, and M. Kirsch-Volders. 1998. Evaluation of the in vitro direct and indirect genotoxic effects of cobalt compounds using the alkaline comet assay. Influence of interdonor and interexperimental variability. Carcinogenesis, 19:2021-2029.

5.8.2 DEVELOPMENTAL TOXICITY

Type	:	Developmental toxicity
Guideline/method	:	Not specified
Species	:	Rat
Strain	:	Wistar
Sex	:	Female
Route of admin.	:	Gastric intubation
Exposure period	:	Gestation day 14 through 21 days of lactation
Frequency of treatment	:	Daily
Duration of test	:	Through lactation day 21
Doses	:	12, 24, and 48 mg/kg b.w. (equivalent to 5.4, 10.8, or 21.8 mg Co/kg b.w.)
Control group	:	Yes
NOAEL maternal tox.	:	Not determined (no maternal data reported)
NOAEL teratogen.	:	Malformations not observed
Other	:	
Other	:	
Other	:	
Year	:	1985
GLP	:	No
Test substance	:	Cobalt chloride
Method	:	
Method detail	:	Cobalt chloride was administered to three groups of 15 pregnant rats from gestation day 14 through the 21st day of lactation. Pups were weighed and examined for signs of toxicity on days 1, 4, and 21 of lactation, and were sacrificed on day 21. Macroscopic examinations were made of the heart, lungs, spleen, liver, and kidneys following sacrifice. Clinical chemistry parameters were also measured.
Result	:	There was significant mortality of pups in the highest dose group and fewer litters produced at all dose levels. In addition, pups showed stunted growth (weight and length) at all dose levels. Relative weights of the liver (males and females) and spleen (females only) were reduced by cobalt exposure, but did not show a dose-related trend. Blood analysis and clinical chemistry showed no treatment related differences. No external malformations were observed in pups. Data from previous studies by the authors suggests that the upper two doses levels were maternally toxic, therefore, the results observed may have been indirectly due, at least in part, to effects on the mothers, rather than direct effects on the fetuses.
Remark	:	
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Domingo, J.L., J.L. Paternain, J.M. Llobet, and J. Corbella. 1985. Effects of cobalt on postnatal development and late gestation in rats upon oral administration. Rev. Esp. Fisiol., 41:293-298.

Type	:	Teratogenicity
Guideline/method	:	Not specified
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Female
Route of admin.	:	Oral gavage
Exposure period	:	Day 6 to 15 of gestation
Frequency of treatment	:	Daily
Duration of test	:	To day 20 of gestation
Doses	:	25, 50, or 100 mg/kg (equivalent to 6.2, 12.4, and 24.8 mg Co/kg b.w.)
Control group	:	Yes

NOAEL maternal tox.	:	Not determined (effects on weight gain seen at lowest dose)
NOAEL teratogen.	:	24.8 mg Co/kg b.w.
Other	:	NOAEL for maternal hematology was 12.4 mg Co/kg b.w.
Other	:	
Other	:	
Year	:	1998
GLP	:	
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	
Method detail	:	Pregnant females (20 per group) were dosed daily with cobalt chloride hexahydrate in distilled water during gestation days 6 to 15. Maternal body weights were recorded on days 0, 6, 9, 12, 16, and 19 of gestation. Individual food consumption was recorded for the following intervals: days 0-6, 6-9, 9-12, 12-16 and 16-19. Detailed physical examinations for signs of toxicity were performed at the same time that weights were recorded. On day 20 of gestation, dams were weighed, then sacrificed. Blood samples were collected for hematological analyses. After exsanguinations, the uterine horns were opened, examinations made and the following recorded: number of corpora lutea, total implantations, number of live and dead fetuses number of resorptions, average fetus body weight, number of stunted fetuses, fetal body length, and fetal tail length. Fetuses were also fixed, stained and examined for skeletal abnormalities.
Result	:	Maternal effects included significant reductions in weight gain and food consumption, particularly at the 24.8 mg Co/kg dose level, although effects on weight gain were found at all dose levels. Hematological parameters (e.g., hematocrit, hemoglobin content) were significantly increased in the highest dose group. No treatment-related changes were observed in the number of corpora lutea, total implants, resorptions, number of live and dead fetuses per litter, fetal size parameters, or fetal sex distribution data. Increased incidences of stunted fetuses per litter (those under two-thirds of the average fetus body weight) were seen in the two highest dose groups; however, the increases were not statistically significant. Examination of fetuses for gross external abnormalities, skeletal malformations, and ossification variations produced negative findings, indicating that cobalt doses as high as 24.8 mg Co/kg do not produce teratogenicity or significant fetotoxicity in the rat.
Remark	:	A lack of teratogenicity in the golden hamster has also been reported (Ferm, V.H. 1972. Adv. Teratol., 6:51-75.
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Paternain, J.L., J.L. Domingo, and J. Corbella. 1988. Developmental toxicity of cobalt in the rat. J. Toxicol. Environ. Health, 24:193-200.

Type	:	Developmental toxicity
Guideline/method	:	Chernoff/Kavlock developmental toxicity screen
Species	:	Mouse
Strain	:	ICR/SIM
Sex	:	Female
Route of admin.	:	Oral intubation
Exposure period	:	Gestation days 8 through 12
Frequency of treatment	:	Daily
Duration of test	:	Through postnatal day 3
Dose	:	180 mg/kg/day (equivalent to 81.7 mg Co/kg)
Control group	:	Yes
NOAEL maternal tox.	:	Not determined
NOAEL teratogen.	:	180 mg/kg/day (equivalent to 81.7 mg Co/kg)
Other	:	

Other	:	
Other	:	
Year	:	1986
GLP	:	
Test substance	:	Cobalt chloride
Method	:	Chernoff, N. and R.J. Kavlock. 1982. J. Toxicol. Environ. Health, 10:541-550.
Method detail	:	The screening test was carried out with a single minimally dose that was expected to result in significant maternal weight reduction, up to 10% mortality, or other clinical sings of overt toxicity. Treatment was by oral intubation on days 8 through 12 of gestation. Mice were allowed to deliver, and neonates examined, counted, and weighed on the day of birth (day 1) and day 3. Dead neonates were recovered from the nest and examined for abnormalities.
Result	:	The average maternal weight gain was significantly affected by cobalt treatment as desired in the protocol. Despite this, there was no effect of cobalt on litter size, percent survival of neonates on days 1-3, or average neonatal weight.
Remark	:	Results are in agreement with those seen in the rat, although another researcher has reported that injections of cobalt chloride to pregnant mice can lead to interference of skeletal ossification in fetuses (Wide, M. 1984. Environ. Res., 33:47-53).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Seidenberg, J.M. D.G. Anderson, and R.A. Becker. 1986. Validation of an in vivo developmental toxicity screen in the mouse. Teratog. Carcinog. Mutagen., 6:361-374.

5.8.3 TOXICITY TO REPRODUCTION

Type	:	Male reproduction
Guideline/method	:	Not specified
In vitro/in vivo	:	In vivo
Species	:	Mouse
Strain	:	CD-1
Sex	:	Male
Route of admin.	:	Drinking water
Exposure period	:	12 weeks (dose-response study); 13 weeks (time course study)
Frequency of treatment	:	Continuous
Duration of test	:	12 weeks (dose-response study); 33 weeks (time course study)
Doses	:	10, 200, or 400 ppm in the dose-response study (equivalent to a daily intake of 23.0, 42.0, or 72.1 mg Co/kg b.w.); 400 ppm in the time course study (equivalent to a daily intake of 58.9 mg Co/kg b.w.)
Control group	:	Yes
Year	:	1988
GLP	:	No
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	
Method detail	:	In the dose-response study, males (5 per dose) were evaluated after 12 weeks of exposure for testicular weight, epididymal sperm concentration, sperm motility, sperm fertilizing ability (fertility), prostatic weight, seminal vesicle weight, and serum levels of testosterone. In the time course study, males (5 per dose and time point) were evaluated after 7, 9, 11, or 13 weeks of exposure for most of these same parameters. In addition, fertility of the males was evaluated at regular intervals up to 20 weeks after cessation of cobalt treatment in the drinking water.
Result	:	Cobalt exposure affected male reproductive parameters in a time- and

		dose-dependent manner. There was a significant decrease in testicular weight and epididymal sperm concentration after 11-13 weeks of exposure at all dose levels. Sperm motility and fertility were significantly depressed in the highest exposure groups. After cessation of exposure, some recovery was seen in fertility over time; however, indices remained significantly depressed through study termination (20 weeks after cessation). Parallel studies with acute cobalt chloride exposures (i.p injections of 200 μmoles/kg for 3 consecutive days) did not result in significant changes in male reproductive parameters, although transient affects on fertility were observed.
Remark	:	Histopathology studies of testes from mice treated with the same general exposure regimen as in this study (i.e., 400 ppm in drinking water for 13 weeks) showed a reproducible, sequential pattern of seminiferous tubule degeneration (Anderson, M.B. et al., 1992. Reprod. Toxicol., 6:41-50). Results of this study are highly consistent with others in which testicular degeneration and atrophy have been reported in rats exposed to 13.2 to 30.2 mg Co/kg/day as cobalt chloride for 2-3 months in the diet or drinking water (ATSDR Sept 2001 Draft).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Pedigo, N.G., W.J. George, and M.B. Anderson. 1988. Effects of acute and chronic exposure to cobalt on male reproduction in mice. Reprod. Toxicol., 2:45-53.
Type	:	Male reproduction
Guideline/method	:	Not specified
In vitro/in vivo	:	In vivo
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male
Route of admin.	:	Diet
Exposure period	:	98 d
Frequency of treatment	:	Continuous in diet
Duration of test	:	Up to 98 d
Doses	:	265 ppm in diet (equivalent to 20 mg Co/kg b.w. at test initiation)
Control group	:	Yes
Year	:	1985
GLP	:	No
Test substance	:	Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_20$)
Method	:	
Method detail	:	Three rats from the control and treatment groups were sacrificed on days 1, 2, 7, 14, 21, 28, 35, 42, 56, 63, 70, 84, and 98. Tissue specimens from the testes, cauda epididymus, and seminal vesicles were fixed and later examined.
Result	:	Dietary cobalt exposure induced consistent degenerative and necrotic lesions in the seminiferous tubules of rats. Cyanosis and engorgement of testicular vasculature on day 35 and thereafter was followed on day 70 by degenerative and necrotic changes in the germinal epithelium and Sertoli cells. Findings indicate that cobalt readily crosses the blood-testes barrier.
Remark	:	Results are consistent with those of Nation et al. (1983), who found significant testicular atrophy in rats exposed chronically to 20 mg Co/kg in the diet (Nation, J.R. et al., 1983. Neurobehav. Toxicol. Teratol., 5:9-15).
Reliability	:	2 (Reliable with restrictions): comparable to guideline study with adequate documentation.
Reference	:	Corrier, D.E., H.H. Mollenhauer, D.E. Clark, M.F. Hare, and M.H. Elissalde. 1985. Testicular degeneration and necrosis induced by dietary cobalt. Vet. Pathol., 22:610-616.

6.0 OTHER INFORMATION

6.1 CARCINOGENICITY

The US National Toxicology Program does not recognize cobalt as a human carcinogen, but IARC has classified cobalt and cobalt compounds as possibly carcinogenic to humans (Class 2B) based on sufficient evidence that cobalt metal powder and cobaltous oxide are carcinogenic in animals (Barceloux 1999, ATSDR Sept 2001 Draft). "No studies were located regarding carcinogenic effects in animals after oral exposure to stable [non-radioactive] cobalt." (ATSDR Sept 2001 Draft).

1. General Information

Id 61789-51-3

Date December 22, 2005

201-16127B

1.0 SUBSTANCE INFORMATION

Generic Name	:	Cobalt naphthenate
Chemical Name	:	Naphthenic acids, cobalt salt (CA index name)
CAS Registry No.	:	61789-51-3
Component Cas Nos.	:	
EINECS No.	:	
Structural Formula	:	$Co(MRCO_2)(NRCO_2)$ Where, R = alkyl group with a chain length of 0 to 10 carbon atoms; M & N are typically one or two fused rings (usually cyclopentane but occasionally cyclohexane or heptane rings) that may contain one or more alkyl substitutions. The total number of carbon atoms in M or N ranges from about 9 to 25. In some cases, no fused ring is present and M or N may be straight-chain or multiple branched carbon/hydrogen/oxygen molecules.
Molecular Weight	:	180 – 350 Cited in EPA (1981); 239 – 409 Cited in EPA (1983); 407 Cited in IPCS (2001).
Synomyms and Tradenames	:	Naftolite
References	:	EPA (1981). Chemical Hazard Information Profile - Draft Report. Cobalt Naphthenate, CAS No. 61789-51-3. U.S. Environmental Protection Agency, Office of Toxic Substances. 8 p. [Subsequently referenced as EPA, (1981)] EPA (1983). Twelfth Report of the Interagency Testing Committee to the Administrator; Receipt of Report and Request for Comments Regarding Priority Lists of Chemicals. U.S. Environmental Protection Agency. Fed. Reg. 48, 24443-24452, June 1. [Subsequently referenced as EPA (1983)] IPCS (2001). International Chemical Safety Card 1093, Cobalt Naphthenate. International Programme on Chemical Safety, 2 p. [Subsequently referenced as IPCS (2001)]

2.1 MELTING POINT

Type	:	
Guideline/method	:	
Value	:	77°C for product with 10.5% Co. Cited in EPA (1981) and EPA (1983); 140°C Cited in IPCS (2001)
Decomposition	:	at °C
Sublimation	:	
Year	:	
GLP	:	
Test substance	:	As a result of production methods, cobalt naphthenate does not exist commercially as a pure chemical substance, but instead is available only in a petroleum-based solvent or a mineral spirit solution. EPA (1984).
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** The melting point range for commercially available naphthenic acids is reported as –35°C to +2ºC (Appendix B). **Metal:** The reported melting point for cobalt chloride is 735ºC (Appendix D).
Reliability	:	[4] Not assignable; Secondary reference sources.
References		EPA (1981); EPA (1983); EPA (1984). Calcium, Cobalt, and Lead Naphthenates: Response to the Interagency Testing Committee. U.S. Environmental Protection Agency. Fed. Reg. 49:21411-21418, May 21. [Subsequently referenced as EPA (1984)]

2.2 BOILING POINT

Type	:	
Guideline/method	:	
Value	:	315 – 380°C (hPa not specified) Cited in EPA (1983); >150°C (hPa not specified) Cited in IPCS (2001).
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	Cobalt naphthenate containing 6% cobalt
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** The boiling point range for commercially available naphthenic acids is reported as 140°C to 200ºC (Appendix B). **Metal:** The reported boiling point for cobalt chloride is 1,049ºC (Appendix D).
Reliability	:	[4] Not assignable; Secondary reference source.
Reference	:	EPA (1983).

2.3 DENSITY

Type	:	
Guideline/method		

Values	:	0.9 for liquid (temperature not specified) Cited in EPA (1981); 0.97 for liquid (temperature not specified) Cited in EPA (1981); 0.91 to 0.95 for liquid with 6% Co (at 77°C) Cited in EPA (1983); 1.16 for solid with 10.5% Co (temperature not specified) Cited in EPA (1981).
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** The reported density of naphthenic acids is 0.91 to 0.96 g/cm^3 (Appendix B). **Metal:** The reported density of cobalt (II) chloride is 3.367 at 25ºC (Appendix D.)
Reliability	:	[4] Not assignable; Secondary reference sources.
Reference		EPA (1981); EPA (1983).

2.4 VAPOR PRESSURE

Type	:	
Guideline/method	:	
Value	:	hPa at °C
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** Estimated vapor pressures of the components of naphthenic acid mixtures would be extremely low (Appendix B).
Reliability	:	
Reference	:	

2.5 PARTITION COEFFICIENT

Type	:	
Guideline/method	:	
Partition coefficient	:	
Log Pow	:	at °C
pH value	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** Estimated log partition coefficient (log Kow) for a range of molecular weight naphthenic acids in Athabasca oil sands extracts ranged from 5.1 to 9.2; lower molecular weights would result in lower log Kow values. (Appendix B). **Metal:** not applicable. Cobaltous chloride dissociates in water.
Reliability	:	

Reference	:	

2.6 SOLUBILITY IN WATER

Type	:	Water solubility determination
Guideline/method	:	OECD 105; EPA OPPTS 830.7840
Value	:	34.28 mg/L at 20ºC
pH Value	:	
Concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at °C
Description	:	
Stable	:	
Deg. Product	:	
Year	:	2003
GLP	:	Yes
Test substance	:	Naphthenic acids, cobalt salt, batch 20031MI, purity ≤65% in mineral spirits, liquid, expiration date December 31, 2003.
Deg. products CAS#	:	
Method	:	OECD 105, Water Solubility, 1995; EPA Product Properties Test Guidelines, OPPTS 830.7840, Water Solubility: Column Elution Method, Shake Flask Method, 1998
Method detail	:	A preliminary test indicated that the column elution method was appropriate. Glass beads (6.06 g) were weighed and placed in a 100 mL round bottom flask. Test item (0.125 g) and dichloromethane (10 mL) were added and the mixture sonicated. The dichloromethane was then evaporated using a gentle stream of nitrogen. The loaded carrier was poured into the elution column which was then filled with water. The system was equilibrated for approximately 2 hours. Elution of the test material from the carrier was performed using a circulation pump. Test temperature was 20ºC. The flow rate was 0.52 mL/min in the first part of the test (about 51 hours) and 0.26 mL/min in the second part of the test (about 24 hours). The apparatus was run until equilibration of the saturation column was obtained, defined by at least five successive samples whose concentrations do not differ more than 30%. The column eluate was sampled at intervals of at least 1 hour to determine the concentration of cobalt, using atomic absorption spectroscopy.
Result	:	Based upon the results of 12 samples, the cobalt solubility was 5.5 mg/L (SD ± 0.4 mg/L) which corresponds to a water solubility of naphthenic acids, cobalt salt of 34.28 mg/L (calculated based on cobalt content of 16.05%).
Remark	:	**Supporting data for dissociation products:** **Acid:** Estimated water solubility for a range of molecular weight naphthenic acids in Athabasca oil sands extracts ranged from 0.0003 to 2.1 mg/L; lower molecular weights would result in higher water solubilities (Appendix B). **Metal:** The water solubility of cobalt (II) chloride was reported to be 450 g/L at 7ºC (Appendix D).
Reliability	:	[1] Reliable without restriction.
Reference	:	Tognucci, A., 2003. Determination of the water solubility of naphthenic acids, cobalt salt. RCC Study No. 849090, conducted for the Metal Carboxylates Coalition by RCC Ltd., Switzerland

2.7 FLASH POINT

Type	:	
Guideline/method	:	

Value	:	121 °C Cited in EPA (1981)
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	[4] Not assignable; Secondary reference source.
Reference	:	EPA (1981).

3.1.1 PHOTODEGRADATION

Type		
Guideline/method	:	
Light source	:	
Light spectrum	:	
Relative intensity	:	based on
Spectrum of substance	:	lambda (max, >295nm) :
		epsilon (max) :
		epsilon (295) :
Conc. of substance	:	at °C
DIRECT PHOTOLYSIS		
Halflife (t1/2)	:	
Degradation	:	% after
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	
Degradation	:	
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	
Deg. products CAS#	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** Naphthenic acids (both mixtures and individual compounds) were not significantly degraded by exposure to natural sunlight, artificial solar radiation, or artificial UV-range radiation (Appendix B). **Metal:** Photodegradation is not applicable for cobalt chloride.
Reliability	:	
Reference	:	

3.1.2 DISSOCIATION

Type	:	Dissociation constant determination
Guideline/method	:	OECD 112
pKa	:	6.74 and 8.00 at 20°C
Year	:	2002
GLP	:	Yes
Test substance	:	Cobalt naphthenate (544574), lot number 20031MI, received from Aldrich Chemical Company. Dark purple liquid, purity of 6.39% cobalt.
Approximate water solubility	:	500 mg/L as determined visually in preliminary study
Method	:	OECD Guideline 112, Dissociation Constants in Water
Method detail	:	Three replicate samples of cobalt naphthenate were prepared at a nominal concentration of 250 mg/L by dissolving 0.0250 grams of test substance in 100 mL of degassed water (ASTM Type II). Each sample was titrated against 0.005 N sodium hydroxide while maintained at a test temperature of 20±1°C. At least 6 incremental additions were made before the first equivalence point and at least 10 incremental additions were made before the second equivalence point. The titration was carried past the final equivalence point. Values of pK were calculated for a minimum of 6 points

		on the titration curve. Phosphoric acid and 4-nitrophenol were used as reference substances.
Result	:	Mean (N = 3) pKa values were 6.74 (SD = 0.0697) and 8.00 (SD= 0.0342) at 20°C. The results indicate that dissociation of the test substance will occur at environmentally-relevant pH values (approximately neutral) and at physiologically-relevant pH values (approximately 1.2).
Remark	:	**Supporting data for dissociation products:** **Acid:** Naphthenic acids exist was weak acids, with most pKa values at about 5 (Appendix B).
Reliability	:	[1] Reliable without restriction.
Reference	:	Lezotte, F.J. and W.B. Nixon, 2002. Determination of the dissociation constant of naphthenic acids, cobalt salts, Wildlife International, Ltd. Study No. 534C-109, conducted for the Metal Carboxylates Coalition.

3.2.1 MONITORING DATA

Type of measurement	:	
Media	:	
Concentration	:	
Substance measured	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Test substance	:	
Reliability	:	
Reference	:	

3.3.1 TRANSPORT (Fugacity)

Type	:	
Media	:	
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Year	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** Using EPIWIN v3.10 Level I fugacity modeling, the principal distribution of naphthenic acids (as found in Athabasca oil sands extracts) would be to soil and/or sediment, with 98% partitioning to soil (Appendix B).
Reliability	:	
Reference	:	

3.4 BIODEGRADATION

Type	:	
Inoculum	:	
Concentration	:	related to related to
Contact time	:	

Degradation	:	(±) % after day(s)
Result	:	
Kinetic of testsubst.	:	% (specify time and % degradation) % % % %
Control substance	:	
Kinetic	:	% %
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	
Deg. products CAS#	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** Commercial mixtures of sodium salts of naphthenic acids degraded when inoculated with microbial populations indigenous to oil sands tailings, with 50% of the organic carbon converted to CO_2 within 24 days; additional studies indicated 60% degradation in 10 days. (Appendix B). **Metal:** Metal does not degrade.
Reliability	:	
Reference	:	

3.5 BIOCONCENTRATION

Type	:	
Guideline/method	:	
Species	:	
Exposure period	:	at °C
Concentration	:	
BCF	:	
Elimination	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

4.1 ACUTE TOXICITY TO FISH

Type	:	
Guideline/method	:	
Species	:	
Exposure period	:	
NOEC	:	
LC0	:	
LC50	:	
LC100	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** The 96-h TLm (LC50) for naphthenic acids for zebra fish (*Brachydanio rerio*) was reported to be 16.3 ppm; for embryos of this species, the 48-h TLm was 3.5 mg/L. The 96-h LC50 for a commercially-available mixture of naphthenic acids for three-spine stickleback (*Gasterosteus aculeatus*) was estimated to be 5 mg/L. The reported 48-h TLm for bluegill (*Lepomis macrochirus*) was 5.6 mg/L. Commercial sodium salts of naphthenic acid produced LC50 values of 50 mg/L for kutum and sturgeon and 75 mg/L for roach. (Appendix B). **Metal:** For cobalt chloride, the 96-h LC50 was 1.41 mg Co/L for the highly sensitive rainbow trout, *Onchorynchus mykiss*. Other fish species are less sensitive with 96-h LC50 values ranging from 22.0 to 333 mg Co/L (Appendix D).
Reliability	:	
Reference	:	

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	
Guideline/method	:	
Species	:	
Exposure period	:	
NOEC	:	
EC0	:	
EC50	:	
EC100	:	
Other	:	
Other	:	
Other	:	
Limit Test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	

Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** A 96-h LC50 of 4.8 mg/L for calcium naphthenate has been reported for the marine copepod, *Nitocra spinipes*. The zooplankton species *Nephargoides maeoticus* tolerated naphthenic acids concentrations only up to 0.15 mg/L (Appendix B). **Metal:** For cobalt chloride, reported 48-h EC50 values for *Daphnia magna* have been reported as 1.52 mg Co/L and as 5.5 mg Co/L. For *Ceriodaphnia dubia*, 48-h LC50 values ranged from 2.35 to 4.60 mg Co/L (Appendix D).
Reliability	:	
Reference	:	

4.3 TOXICITY TO AQUATIC PLANTS (e.g., Algae)

Type	:	
Guideline/method	:	
Species	:	
Endpoint	:	
Exposure period	:	
NOEC	:	
LOEC	:	
EC0	:	
EC10	:	
EC50	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** The toxicity of naphthenic acids to populations of the freshwater diatom, *Navicula seminulum*, has been measured. The 96-h EC50 for growth ranged from 30.5 – 80.5 mg/L (Appendix B). **Metal:** For cobalt chloride, the 96-h EC50 for *Chlorella vulgaris* was 0.52 mg/L. For the duckweed *Lemna minor*, the 7-d IC50 was16.9 mg Co/L, while for the blue-green alga *Spirulina platensis* the 96-h EC50 was 23.8 mg Co/L (Appendix D).
Reliability	:	
Reference	:	

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo	:	In vivo
Type	:	Absorption and disposition
Guideline/method	:	None
Species	:	Fischer 344 rat
Number of animals	:	3 per group; 24 per dose
Males	:	Not specified
Females	:	Not specified
Doses	:	0.333, 3.33, or 33.3 mg Co(II)/kg
Vehicle	:	Ethanol:Emulphor
Route of administration	:	Gavage (single oral dose)
Exposure time	:	0.5, 2, 4, 8, 12, 18, 24, or 36 h
Product type guidance	:	
Decision on results on acute tox. tests	:	Doses ranged from approximately 0.001 to 0.1 times the LD50
Adverse effects on prolonged exposure	:	
Half-lives	:	1^{st}: 1.09 h (intermediate dose); 0.94 h (high dose) 2^{nd}: 6.09 h (intermediate dose); 4.86 h (high dose) 3^{rd}: 24.68 h (intermediate dose); 23.97 h (high dose)
Toxic behaviour	:	
Deg. product	:	
Deg. products CAS#	:	
Year	:	1999
GLP	:	Not specified
Test substance	:	Cobalt naphthenate (>99.6% purity; 11.9% cobalt)
Method	:	Rats were assigned to one of eight groups that were sacrificed 0.5 to 36 h after dosing. Tissues, urine and feces were collected over a 36-h period from the low- and high-dose groups; blood was collected from all 3 dose groups. Analyzed tissues included heart, liver, kidney, spleen, testes, small intestine, large intestine, stomach, and contents of the intestines and stomach. Cobalt in tissue, blood, and excreta samples was analyzed by graphite furnace atomic absorption spectroscopy.
Method detail	:	Determination of absorption and disposition for the naphthenic acid moiety was not attempted.
Result	:	The majority of the dose in both the low- and high-dose groups was excreted in the feces (42 and 73.1%, respectively), indicating that cobalt was incompletely absorbed from the gastrointesinal tract following oral dosing. The percent of the dose excreted in the urine was similar for low and high doses (31.8% and 26.3%, respectively). Cobalt concentrations were found to be highest in the liver and kidneys. The peak plasma concentrations of 0.6 and 1.7 μg Co/ml occurred at approximately 4.3 h after dosing for the intermediate-dose group, and 3.3 h after dosing for the high-dose group. Blood concentration curves were triphasic and clearly showed absorption and elimination phases. The terminal half-lives were 24.7 and 24 h for the intermediate- and high-dose groups, respectively. The extent of urinary excretion of cobalt in this study was similar to that found previously for an equivalent dose of cobalt in the form of cobalt chloride. Study data and previous dissolution study results indicate that cobalt can dissociate from cobalt naphthenate when administered orally and subjected to acidic conditions in vivo. Under these conditions, the naphthenic acid moiety appeared to have no influence on uptake of cobalt.
Remark	:	**Supporting data for dissociation products:** **Metal**: Absorption of cobalt in the digestive tract is influenced by the

chemical form of the metal. The soluble form, cobalt chloride, is absorbed 13-34% in the gut of rats, but absorption in the gut may be increased in iron deficient individuals. The highest concentration of absorbed cobalt is in the liver and then the kidney. There is no accumulation of cobalt with age. Following oral exposure, cobalt is eliminated primarily in feces and secondarily in urine. For the more soluble forms of cobalt, e.g., cobalt chloride, 70 – 80% of the administered dose is eliminated in the feces. For absorbed cobalt, elimination is rapid primarily in the urine. Elimination is biphasic or triphasic. The terminal phase involves a very small residual level of cobalt and has a half-life in years (Appendix D).

Reliability : [2] Reliable with restrictions. Acceptable, well-documented publication which meets basic scientific principles.

Reference : Firriolo, J.M., F. Ayala-Fierro, I.G. Sipes, and D.E. Carter. 1999. Absorption and disposition of cobalt naphthenate in rats after a single oral dose. J. Toxicol. Environ. Health. Part A. 58: 383-395.

Additional Reference for Absorption:

Handy, R.W., D.A. Binstock, and D.B. Feldman. 1985. Absorption studies on cobalt and lead naphthenates from commercial products in rats. Research Triangle Institute. NIOSH-0018887. This study investigated the absorption of cobalt in rats after dermal administration (944 mg/kg for up to 5 days) of a oleoresinous paint containing 0.5% cobalt naphthenate. None of the rats had cobalt blood levels above the detection limit (equivalent to 14 μg Co/100 ml), indicating that cobalt in this form is poorly absorbed by the dermal route of exposure.

5.1.1 ACUTE ORAL TOXICITY

Type	:	Oral LD50
Guideline/Method	:	
Species	:	Rat
Strain	:	Sprague-Dawley (albino)
Sex	:	Male and female
Number of animals	:	5 per dose (10 of each sex with either 2 or 3 of each sex per dose)
Vehicle	:	Corn oil
Doses	:	2000, 2510, 3160, and 3980 mg/kg
LD50	:	2,800 mg/kg
Year	:	1973
GLP	:	Not specified
Test substance	:	Monsanto product CP 60809 (Cobalt naphthenate; percentage of cobalt was not completely legible in the document available, but is believed to be 11%)
Method	:	Single oral dose administered via stomach tube.
Method detail	:	Sample fed as a 50% solution in corn oil. No control group. Study conducted by Younger Laboratories, St. Louis, MO. (Monsanto project number Y-73-18)
Result	:	No mortality (0/5) at 2000 mg/kg; 20% mortality (1/5) at 2510 mg/kg; 60% (3/5) mortality at 3160 mg/kg; 100% mortality (5/5) at 3980 mg/kg. Survival time was one to four days, with most deaths occurring within two days. Toxic signs included reduced appetite and activity, increasing weakness, collapse, and death. At autopsy there was slight lung congestion, liver discoloration, and acute gastrointestinal inflammation.
Remark	:	**Supporting data for dissociation products:** **Acid:** The acute oral LD50 for naphthenic acids has been reported as 5.88 g/kg; other data for rats indicates LD50 values of 3.0 – 5.2 g/kg. The oral LD50 for mice was reported as 3.55 g/kg (Appendix B). **Metal:** For cobalt chloride hexahydrate, the oral LD50 in rats was 766

mg/kg (equivalent to 190 mg Co/kg). Other reported LD50s range from 19.8 to 85.5 mg Co/mg bw in rats. In mice, the LC50 for cobalt chloride waas reported as 89.3 mg Co/kg bw were reported (Appendix D).

Reliability : [2] Reliable with restrictions; Basic data given: comparable to guidelines.
Reference : Younger Laboratories, Inc. 1973. Certificate of Analysis. Cobalt Naphthenate. Toxicological Investigation of CP 60809. Monsanto Project Number Y-73-18. [Available from the National Technical Information Service in microfiche OTS0545989, "Initial submission: toxicological investigation of: CP 60809 with cover letter dated 081892" and in microfiche OTS0206554, „Toxicological investigation of: CP 60809 with cover letter"][Subsequently referenced as Younger Labs (1973)]

Type : Oral LD50
Guideline/Method :
LD50 : 2,828 mg/kg (95% confidence limit of 1607 to 4978 mg/kg)
Species : Rat (young adult)
Strain : ChR-CD
Sex : male
Number of animals : 5 per dose
Vehicle : Peanut oil
Doses : 1000, 2000, and 4000 mg/kg
Year : 1968
GLP : Not specified
Test substance : Cobalt naphthenate (manufactured by K & K Laboratories, Inc., Plainview, NY)
Method : Single oral dose administered via stomach tube.
Method detail : Sample fed as a 25% solution in peanut oil. No control group. Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, E.I. Du Pont de Nemours and Company.
Result : No mortality (0/5) at 1000 mg/kg; 20% mortality (1/5) at 2000 mg/kg; 80% (4/5) mortality at 4000 mg/kg.
Remark : LD50 value is almost identical to that determined above for Sprague – Dawley rats by Younger Labs (1973).
Reliability : [2] Reliable with restrictions; Basic data given: comparable to guidelines.
Reference : Du Pont (1983). Health and Safety Data Reporting: ITC Twelfth Report. [Available from the National Technical Information Service in microfiche OTS0215503, "Oral LD50 test with cover letter"] [Subsequently referenced as Du Pont (1983)]

Type : Oral LD50
Guideline/Method :
LD50 : 3.9 g/kg (range 3.5 to 4.4 g/kg)
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle : Stoddard-type solvent
Doses :
Year :
GLP :
Test substance : Cobalt naphthenate containing 6.0% cobalt
Method : Dosing by gavage
Method detail :
Result : Death appeared to result from gastrointestinal disturbances. Death was not sudden and typically occurred 3 to 4 days after administration.
Remark : The LD50 in this study is equivalent to 234 mg Co/kg. As measured by the

		LD50, the cobalt salt was more toxic than the calcium (>6.0 g/kg), copper (>6.0 g/kg), lead (5.1 g/kg), manganese (>6.0 g/kg), and zinc (>6.0 g/kg) salts of naphthenic acid, and was similar in toxicity to one naphthenic acid fraction derived from crude kerosene acids (3.0 g/kg) that was also tested. A phenyl mercury naphthenate (10% Hg) was considerably more toxic than all of these compounds with an LD50 of 0.39 g/kg.
Reliability	:	[4] Not reliable. Documentation insufficient for assessment.
Reference	:	Rockhold, W.T. 1955. Toxicity of naphthenic acids and their metal salts. A.M.A. Arch. Indust. Health. 12: 477-482.

5.1.2 ACUTE INHALATION TOXICITY

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	
LC50	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Metal:** No acute inhalation toxicity studies were located for cobaltous chloride (Appendix D).
Reliability	:	
Reference	:	

5.1.3 ACUTE DERMAL TOXICITY

Type	:	Minimum lethal dose
Guideline/method	:	
Species	:	Rabbit
Strain	:	New Zealand white (albino)
Sex	:	Male and female
Number of animals	:	One per dose (male and female alternated)
Vehicle	:	None
Doses	:	794, 1260, 2000, and 5010 mg/kg
LD50	:	>1260 mg/kg; <2000 mg/kg
Year	:	1973
GLP	:	Not specified
Test substance	:	Monsanto product CP 60809 (Cobalt naphthenate; percentage of cobalt was not completely legible in the document available, but is believed to be 11%)
Method	:	Skin (dermal) absorption
Method detail	:	Substance was applied undiluted to the closely clipped, intact skin. The treated areas were covered with plastic strips and the animals held in wooden stocks for periods up to 24 hours, after which time they were assigned to individual cages. Survival was evaluated after 14 days.

Result	:	Rabbits dosed at 794 and 1260 mg/kg survived. Rabbit (female) dosed at 2000 mg/kg died after 4 days. Rabbit (male) dosed at 5010 mg/kg died after 3 days. Toxic signs included reduced appetite and activity (three to five days in survivors), increasing weakness, collapse, and death. At autopsy there was liver discoloration, enlarged gall bladder, and gastrointestinal inflammation.
Remark	:	**Supporting data for dissociation products:** **Acid:** The LD50 for rabbits dermally exposed to raw naphthenic acid derived from kerosene was >3.16 g/kg (Appendix B). **Metal:** Increased proliferation of lymphatic cells was seen in mice and guinea pigs dermally exposed to cobalt chloride, with LOAEL values ranging from 9.6 to 14.7 mg Co/kg/day. (Appendix D).
Reliability	:	[2] Reliable with restrictions; Basic data given: comparable to guidelines.
Reference	:	Younger Labs (1973).

5.2.1 SKIN IRRITATION

Type	:	Skin irritation
Guideline/method	:	
Species	:	Rabbit
Strain	:	New Zealand white (albino)
Sex	:	Male and female
Concentration	:	Undiluted
Exposure	:	
Exposure time	:	24 hr
Number of animals	:	Three
Vehicle	:	None
Classification	:	Slight irritant
Year	:	1973
GLP	:	Not specified
Test substance	:	Monsanto product CP 60809 (Cobalt naphthenate; percentage of cobalt was not completely legible in the document available, but is believed to be 11%)
Method	:	Dermal irritation
Method detail	:	Substance was applied undiluted to the closely clipped, intact skin and covered with a one inch square patch, two single layers thick. Patches were held in place for 24 hours with adhesive tape. Observations were made over a period of seven days for irritation. Data were scored according to the method of Draize et al., 1944.
Result	:	The compound was classified as a slight irritant when applied undiluted. The average maximum score was 2.0 out of a possible 8 after an exposure time of 24 hours. Irritation cleared within 72 hours. Due to the deep color of the sample, no erythema score was given. Observations: 1–hr: No skin changes; score of zero 24 hr: Slight edema; no erythema; score of 2.0 48 hr: Very slight edema in two animals; no erythema; score of 0.6 72 to 168 hr: No erythema or edema; score of zero
Remark	:	**Supporting data for dissociation products:** **Acid:** Raw naphthenic acid derived from kerosene was moderately to severely irritating to rabbits (Appendix B). **Metal:** Dermatitis is a common result of dermal exposure to cobalt in humans and is probably caused by an allergic reaction (Appendix D).
Reliability	:	[2] Reliable with restrictions; Basic data given: comparable to guidelines.
Reference	:	Younger Labs (1973).

5.2.2 EYE IRRITATION

Type	:	Eye irritation
Guideline/method	:	
Species	:	Rabbit
Strain	:	Albino
Sex	:	Male and female
Concentration	:	Undiluted
Dose	:	0.1 mL
Exposure time	:	24 hr
Number of animals	:	Three (2 male; 1 female)
Vehicle	:	None
Classification	:	Slight irritant
Year	:	1973
GLP	:	Not specified
Test substance	:	Monsanto product CP 60809 (Cobalt naphthenate; percentage of cobalt was not completely legible in the document available, but is believed to be 11%)
Method	:	Eye irritation
Method detail	:	0.1 mL of undiluted sample was placed in the conjunctival sac of the right eye of each of three albino rabbits. The left eye served as a control. The eyes were rinsed with warm isotonic saline solution after 24 hours (following the 24-hr observation). Observations were made over a period of 7 days for inflammation. Data were scored according to the method of Draize et al.
Result	:	The compound was classified as a slight eye irritant. The average maximum score peaked at 12.6 out of a possible 110 at the 24-hr time point. Scores ranged from 7.3 at 1 hour to zero at 120 hours and thereafter. Observations at 24 hours included moderate erythema, very slight to slight edema, and copious discharge containing whitish exudate.
Remark	:	**Supporting data for dissociation products:** **Acid:** Raw naphthenic acid derived from kerosene was judged to be a moderate eye irritant to rabbits (Appendix B).
Reliability	:	[2] Reliable with restrictions; Basic data given: comparable to guidelines.
Reference	:	Younger Labs (1973).

Type	:	Eye irritation
Guideline/method	:	
Species	:	Rabbit
Strain	:	Albino
Sex	:	
Concentration	:	Undiluted powder; 10% suspension in propylene glycol; 6% in mineral spirits; or 0.15% in cyclohexane
Dose	:	10 mg of powder or 0.1 mL of liquid
Exposure time	:	20 seconds if eye was washed; up to several days if eye was unwashed
Number of animals	:	2 per treatment
Vehicle	:	Propylene glycol, mineral spirits, cyclohexane, or none
Classification	:	Mild transient irritant
Year	:	1966
GLP	:	Not specified
Test substance	:	Cobalt naphthenate (percent of cobalt not specified) as powder or liquid
Method	:	Eye irritation
Method detail	:	Each test material was instilled into one conjunctival sac of each of two albino rabbits (10 mg of powdered test substance or 0.1 mL of liquid substances). The other eye of each rabbit served as an untreated control. After 20 seconds of contact, one of the two treated eyes was washed with tap water for a 1-minute interval. The other eye of this rabbit was not

washed. Observations were made with a band slit lamp at one and four hours and at one, two and three days, or until eyes were normal. Examinations after the day of treatment were made with the aid of 5% fluorescein and a biomicroscope.

Result : None of the treatments produced effects on the cornea. The 10% suspension in propylene glycol caused a slight injection of the blood vessels in the iris, but this effect did not persist for more than 4 hours. All treatments produced mild transient conjunctival irritation (i.e., redness and discharge), which was more pronounced in the case of the propylene glycol suspension. Ocular effects cleared within 1 to four days after treatment. Washing the eyes with water did not appreciably lessen the irritation.

Remark :

Reliability : [2] Reliable with restrictions; Basic data given: comparable to guidelines.

Reference : Du Pont (1983).

5.3 REPEATED DOSE TOXICITY

Type :
Guideline/method :
Species :
Strain :
Sex :
Route of admin. :
Exposure period :
Frequency of treatm. :
Post exposure period :
Doses :
Control group :
NOAEL :
LOAEL :
Other :
Year :
GLP :
Test substance :
Method :
Method detail :
Result :
Remark : **Supporting data for dissociation products:**
Acid: An oral 90-d subchronic toxicity test with a mixture of naphthenic acids (sodium salts) isolated from Athabasca oil sands produced significant physical, clinical, and pathological changes n rats at a dose level of 60 mg/kg/day (5 doses per week). No significant adverse effects were seen at a dose level of 6 mg/kg/day. Several parameters suggested that the liver was the primary target organ in this study. Liver weight was increased 35% above control values in the high dose group. Body weight gain was also reduced 8-9% in this exposure group compared to controls. (Appendix B).
Metal: Repeated oral dosing of rats with cobalt chloride hexahydrate for 8 weeks indicated the NOAEL was 0.6 mg Co/kg and the LOAEL was 2.5 mg Co/kg, based upon changes in hemoglobin content and numbers of erythrocytes. Another study reported oral doses of 0.5 and 2.5 mg Co/kg for 7 months stimulated hematopoiesis and decreased immunological reactivity in rats, while doses of 0.05 mg Co/kg had no effects. (Appendix D).

Reliability :
Reference :

5.4 GENETIC TOXICITY 'IN VITRO'

Type	:	Mutagenicity
Guideline/method	:	Ames assay
System of testing	:	Bacteria *in vitro*
Test concentrations	:	100 – 10,000 µg/plate
Species		*Salmonella typhimurium*
Strains		TA98, TA100, TA1537, TA1538
Cycotoxic concentr.	:	
Metabolic activation	:	Conducted both with and without activation. Activation system consisted of rat or hamster liver S-9 fraction induced with Aroclor 1254 (10%).
Year	:	
GLP	:	
Test substance	:	Cobalt naphthenate
Method	:	Standard plate
Method detail	:	DMSO used as solvent
Result	:	Negative for all strains and test combinations except strain TA98 using a rat liver S-9 fraction.
Remark	:	Cobalt naphthenate was negative in the *Salmonella* study for all strains (TA97, TA98, TA100 and TA1535) both with and without activation using rat and hamster liver S-9 (National Toxicology Program, Study ID A28503, http://ntp-server.niehs.nih.gov). **Supporting data for dissociation products:** **Acid:** The calcium, sodium, and copper salts of naphthenic acid were negative in the *Salmonella* mutagenicity test (Appendix B). **Metal:** Cobalt compounds with a valence state of II, the form of cobalt released by dissociation of cobalt chloride, are generally non-mutagenic in bacterial assays, including plate incorporation and fluctuation assays with *Salmonella typhimurium* TA strains and *Escherichia coli* WP2. However, a weak positive mutagenic response has been found in the rec assay with *Bacillus subtilis* and in Chinese hamster V9 cells. DNA damage in isolated human lymphocytes was observed at 6.0 mg Co/L in the alkaline comet assay, and an increase in sister chromatid exchanges has been observed in human lymphocytes and macrophages (Appendix D).
Reliability	:	[2] Reliable with restrictions.
Reference	:	Short-term test program sponsored by the Division of Cancer Biology, National Cancer Institute, Dr. Shen Yang, Project Officer. (Cited In Chemical Carcinogenesis Research Information System, National Library of Medicine: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS) [Subsequently referenced as NCI (undated)]

Type	:	Mutagenicity
Guideline/method	:	
System of testing	:	Mouse lymphoma
Species		Mouse
Strain		L5178Y (TK+/TK-)
Test concentrations	:	0.01 - 0.049 µl/ml (without metabolic activation) 0.03 – 0.17 µl/ml (with metabolic activation)
Cycotoxic concentr.	:	
Metabolic activation	:	Conducted both with and without activation. Activation system consisted of rat liver S-9, induced with Aroclor 1254.
Year	:	
GLP	:	
Test substance	:	Cobalt naphthenate
Method	:	Suspension/plate
Method detail	:	Acetone used as solvent

Result	:	Negative both with and without activation
Remark	:	**Supporting data for dissociation products:** **Acid:** Similar mouse lymphoma tests with the calcium and copper salts of naphthenic acids were also positive both with and without metabolic activation. (Reference: Short-term test program sponsored by the Division of Cancer Biology, National Cancer Institute, Dr. Shen Yang, Project Officer. Cited in Chemical Carcinogenesis Research Information System, National Library of Medicine: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS). The sodium salt of naphthenic acid produced negative results for chromosome aberrations and positive results for sister chromatid exchanges (Appendix B).
Reliability	:	[2] Reliable with restrictions.
Reference	:	NCI (undated)

Additional Reference for Genetic Toxicity In Vitro:

Crebelli, R. et al. (1985). Br. J. Ind. Med. 42:481. Article reports that cobalt naphthenate (purity unknown) is not mutagenic to *Salmonella typhimurium* bacteria in the Ames test at concentrations from 500 to 2500 µg/plate when conducted with metabolic activation (rat S9 fraction).

5.5 GENETIC TOXICITY 'IN VIVO'

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Route of admin.	:	
Exposure period	:	
Doses	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Metal:** Oral administration of cobalt chloride hexahydrate to mice (20 – 80 mg.kg bw) produced a concentration-dependent increase in chromosomal aberrations. A dose-dependent increase in the incidence of micronucleated polychromatic erthythrocytes was observed in mice subsequent to i.p. injection of $CoCl_2.6H_20$, at doses of 25 – 90 mg Co/kg bw. Increased micronucleus formation was observed following i.p. injection of 12.4 and 22.3 mg Co/kg (as cobalt chloride), but not after injection of 6.19 mg Co/kg (NOEL). (Appendix D).
Reliability	:	
Reference	:	

5.6 DEVELOPMENTAL TOXICITY

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Route of admin.	:	

Exposure period	:	
Frequency of treatm.	:	
Duration of test	:	
Doses	:	
Control group	:	
NOAEL maternal tox.	:	
NOAEL teratogen.	:	
Other	:	
Other	:	
Other	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** In rats orally dosed with naphthenic acids isolated from Athabasca oil sands tailings, no fetal malformation were noted at doses up to 60 mg/kg bw (Appendix B). **Metal:** In a developmental toxicity study with cobalt chloride exposure (5.4 to 21.8 mg Co/kg/day) in rats from gestation day 14 to lactation day 21 the LOAEL was based on stunted pup growth. However, maternal toxicity was observed in conjunction with effects on the offspring. This growth effect was considered to be a secondary or indirect effect rather than a direct effect of cobalt on the fetus. No teratogenic effects were observed. Another study in rats provided a NOAEL of 24.8 mg Co/kg/day for cobalt chloride exposure from gestation days 6-15. No effects were observed on fetal growth or survival in mice exposed to 81.7 mg Co/kg/day as cobalt chloride during gestation days 8-12 (Appendix D).
Reliability	:	
Reference	:	

5.7 TOXICITY TO REPRODUCTION

Type	:	
Guideline/method	:	
In vitro/in vivo	:	
Species	:	
Strain	:	
Sex	:	
Route of admin.	:	
Exposure period	:	
Frequency of treatm.	:	
Duration of test	:	
Doses	:	
Control group	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** In a one-generation reproduction study in male rabbits, no adverse effects on reproductive performance were observed as a result of dermal

exposure of the males to an over-based calcium naphthenate in mineral oil. No signs of toxicity, either systemically or at the treated site, were observed and there were no pathological findings of the reproductive tract. In an oral study with rats, doses of 60 mg/kg/day resulted in dramatic reductions in female fertility and in total cholesterol, while mating and ovulation were unaffected (Appendix B).
Metal: Cobalt exposure (as cobalt chloride hexahydrate in drinking water for 12 -13 weeks) affected male reproductive parameters for mice in a time- and dose-dependent manner. All dose levels (23.0 – 72.1 mg Co/kg-day) caused decreases in testicular weight and epididymal sperm concentration. Testicular degeneration and atrophy have been reported in rats exposed to 13.2 to 30.2 mg Co/kg/day as cobalt chloride for 2-3 months in the diet or drinking water. (Appendix D).

Reliability :
Reference :

Other Information

Carcinogenicity

The studies of Nowak (1961, 1965, 1966) on cobalt naphthenate have been summarized in a BIBRA Toxicity Profile (1999), a cancer review for cobalt compounds (Jensen and Tuchsen, 1990), and a hazard profile for cobalt naphthenate (EPA, 1981). These are the studies with mice and rabbits that could not be evaluated by IARC. Nowak injected rabbits with cobalt naphthenate (about 2 mg of Co) either intramuscularly (i.m.), intrapleurally, intravascularly, or intrahepatically. Injections were given either two or four times per week for two to four months. Nine of 12 rabbits developed tumors at the injection site, including all five rabbits injected intramuscularly. It is unclear whether proper sham-treated controls were include in this study, making study interpretation problematic. In studies with mice, Nowak injected a single dose (0.02 ml) of cobalt naphthenate (7% Co) intramuscularly. This dose is equivalent to about 800 mg/kg. Eight of the 30 mice given injections developed tumors at the injection site within one to three months. No information on control mice was reported. Because of the deficiencies in these studies and the routes of administration used, the Nowak studies are not considered reliable or relevant for characterizing the potential carcinogenic hazard of cobalt naphthenate. A similar conclusion was reached by the EPA in its response to the Interagency Testing Committee (EPA, 1984).

Supporting data for dissociation products:
Acid: In a study in which calcium naphthenate was dermally administered to female mice (two times per day for two years), twelve epidermal and one dermal tumor at the treated sites were observed in eight of the exposed mice. Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present (Appendix C).

Metal: The US National Toxicology Program does not recognize cobalt as a human carcinogen, but IARC has classified cobalt and cobalt compounds as possibly carcinogenic to humans (Class 2B) based on sufficient evidence that cobalt metal powder and cobaltous oxide are carcinogenic in animals. "No studies were located regarding carcinogenic effects in animals after oral exposure to stable [non-radioactive] cobalt." (ATSDR Sept 2001 Draft; see Appendix D).

References:

BIBRA (1999). Toxicity profile cobalt naphthenate TNO BIBRA International, Carshalton, Surrey, UK.

IARC (1991). Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 52. Chlorinated drinking-water; chlorination by-products; some other halogenated compounds, cobalt and cobalt compounds. International Agency for Research on Cancer, Lyon, France. World Health Organization. 363 p.

Jensen, A.T. and F. Tuchsen (1990). Cobalt exposure and cancer risk. Crit. Rev. Toxicol., 20:427-437.

EPA (1981). Chemical Hazard Information Profile - Draft Report. Cobalt Naphthenate, CAS No. 61789-51-3. U.S. Environmental Protection Agency, Office of Toxic Substances. 8 p.

EPA (1984). Calcium, Cobalt, and Lead Naphthenates: Response to the Interagency Testing Committee. U.S. Environmental Protection Agency. Fed. Reg. 49:21411-21418, May 21.

Toxicity Reviews

BIBRA (1999). Toxicity profile cobalt naphthenate (1999). TNO BIBRA International, Carshalton, Surrey, UK.

EPA (1981). Chemical Hazard Information Profile - Draft Report. Cobalt Naphthenate, CAS No. 61789-51-3. U.S. Environmental Protection Agency, Office of Toxic Substances. 8 p.